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STUDIES ON THE HOST-PARASITE RELATIONSHIP OF *NEMATOSPIROIDES DUBIUS* IN THE MOUSE

THESIS

for the

Degree of Doctor of Philosophy

by

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To Carrie with Love

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SUMMARY

The work presented in this thesis has shown that N. dubius has a marked immunosuppressive effect on the expulsion of T. spiralis from mice which was manifested by a delay in the rejection of T. spiralis.

Using cell transfer techniques it was shown that both priming and expression of mesenteric lymph node cells was affected by N. dubius. Experiments designed to detect suppressor cell activity in this system provided equivocal results. The localization of labelled lymphoblasts was also examined in N. dubius and T. spiralis infected mice and it was found that the enhanced homing of lymphoblasts to the small intestine of T. spiralis infected mice was delayed by 8 days when a concurrent N. dubius infection was present.

The ability of mice to mount a peripheral (subcutaneous) inflammatory response to a biologically inert filter was also investigated. No differences were detected in the numbers or rate of accumulation of macrophages or polymorphs onto the filters. An attempt to examine macrophage activity histochemically during N. dubius infection was found to be of little value. Responses of mesenteric lymph node cells from N. dubius infected mice to PHA were depressed throughout the first forty days of infection but it proved impossible to link these results to the measurements obtained from 'in vivo' blastogenesis. Concentrated ES-products from N. dubius adult worms were not toxic for lymphoid cells.

In the second section the immune response against N. dubius was examined. Attempts to transfer immunity with serum obtained from multiply immunized mice were relatively unsuccessful giving a maximum protection against challenge infection of only 26%.

Mesenteric lymph node cell and spleen cell transfers also gave limited protection. Splenectomy had little effect on the survival of a primary infection of N. dubius and did not alter the response to a challenge infection. As a result of the problems encountered with cell and serum transfers other methods of stimulating a transferable immunity were undertaken. The survival of irradiated larvae was examined and it was found that female worm fecundity was abolished by irradiation at 6.5k.rads and that a reduction in worm recoveries could be brought about by doses of 10k.rads and above. At 25k.rads virtually no larvae reached the adult stage. Irradiated larvae were shown to stimulate high levels of protection against challenge infection and their effectiveness was found to be mouse strain independent.

N. dubius adult worms are not normally expelled from the intestine of NIH mice during infection. However, N. dubius adult worms, surgically implanted in low numbers (50 worms) were expelled from the intestines of mice which were in the process of eliminating a T. spiralis infection.

The field strain of N. dubius isolated from A. sylvaticus was not capable of surviving in NIH mice without immunosuppressive treatment. However field strain parasites immunized mice against a challenge infection of normal larvae more effectively than did normal larvae.

ABBREVIATIONS

B	thymus independent; antibody producing
BCG	Bacillus Calmette Guerrin
C	control, (control serum)
CA	cortisone acetate
FCS	Foetal calf serum
Fig.	Figure
G A L T	Gut associated lymphoid tissue
HBSS	Modified Hanks' balanced salt solution
I	immune serum
% ID	Radioactivity in an organ as percentage of the injected dose of isotope
Ig	Immunoglobulin
i p	intraperitoneal(ly)
^{125}I-Udr	(^{125}I)-iodo-deoxyuridine
i/v	intravenous(ly)
K	Kill
K.rad(s)	Kilorad(s)
Liv	Liver
MØ	macrophage
MLN	mesenteric lymph node
MLNC	mesenteric lymph node cells
MWR	Mean worm recovery
Nd	Nematospiroides dubius
P	Probability
PHA	Phytohaemagglutinin
p.i.	post infection
PP	Peyer's patches
PWM	Pokeweed mitogen

ABBREVIATIONS (continued)

PYR	Pyrantel embonate
SC	spleen cells
s/c	subcutaneous(ly)
SD	standard deviation
SI	small intestine
SPL	Spleen
SRBC	Sheep red blood cells
T	Thymus dependent
% TR	Radioactivity in an organ as percentage of the total isotope recovered from the small intestine, mesenteric lymph, liver and spleen.
Tsp	Trichinella spiralis
w/v	weight/volume ratio
1	primary infection
2	secondary (challenge) infection

ES-products Excretory-secretory products

GENERAL INTRODUCTION

Demographic data for 1975 showed that while the world population was 3967 millions there were 4498 million helminthic infections in man, 75% of which were caused by intestinal nematodes (Peters 1978). Although not causing as much morbidity as the Schistosomes or Filariae, these parasites may chronically impair the health of the majority of people who harbour them. Such infections are particularly common in the Tropics where the climate is ideally suited to their transmission. Here the people also have to contend with periodic outbreaks of clinical nematodiasis in their domestic livestock; resulting in loss of production and creating further problems.

In recent years a number of safe and effective anthelminthics, have been introduced which, together with improved methods of animal husbandry, have resulted in the substantial control of gastrointestinal parasites in western countries. However, the limited availability, and more important, high cost of these drugs have restricted their use in the underdeveloped areas where they are needed.

The search for alternative methods of controlling nematode parasites has centred around the immunological aspects of infection. It is generally accepted that a better understanding of the immunology and pathology of diseases will contribute towards a solution of the problems they present. Of course to be effective any solution must make full use of all available methods including anthelminthics, husbandry, sanitation and vector control. One form of control which has been successful in the past and holds even greater potential for the future is the prevention of Cestode, Nematode and Trematode diseases by immunisation. Some of the ways in which immunisation has and might make a contribution are outlined briefly below.

Throughout their life cycle intestinal parasites bombard the immune system of the host with a multiplicity of antigens. Attempts to confer protection have used a variety of worm preparations as a means of introducing these antigens into the host.

On the assumption that 'protective antigens' must originate somewhere within the parasite, killed organisms or homogenates of worms, have been tested as vaccines in experimental animals. These 'dead vaccines', the crudest form of antigen preparation, have in the majority of cases failed to protect (see Review Clegg and Smith 1980). Soluble antigens derived from parasites have also been used in immunisation. The main work in this area has been concerned with antigens which are believed to be involved in penetration and feeding. Obviously any host response acting against these products would impair or prevent parasite establishment and/or survival. Such 'soluble antigens' have been successful particularly with the Trichuroid nematodes T. spiralis and T. muris (Despommier and Muller 1970, Jenkins and Wakelin 1977). In these cases the source of the soluble antigens was in the stichosome, a gland structure comprising of some 30-40 cells, stichocytes which contain large numbers of secretory granules. Despommier and Muller (1970) used isolated granules to immunize animals and found protection ranging from 80 to 90%. Jenkins and Wakelin (1977) found that a stichosomal secretion was extremely efficient (90% protection) at immunizing mice. Protective antigens are not always as easy to locate, however, similar success has been reported by Rothwell (1978) and Stromberg and Soulsby (1977) who found that soluble antigens of Trichostrongylus colubriformis and Ascaris suum protected guinea pigs against homologous challenge. Unfortunately these successes have been in laboratory animals only, and have not been extended to other systems and to clinical trials.

One notable exception to this is the protection of calves against Taenia saginata using parasite antigens obtained from in vitro culture (Rickard and Adelph 1978). Now that more sophisticated separation techniques are available, enabling the isolation and concentration of the important host protective antigens many more vaccines will probably be developed.

In many laboratory models of helminth infections the best protection obtained has been through prior exposure of the host to a completely normal infection. This of course has many disadvantages because of the damage which some of these parasites can inflict on the host; immunity is achieved, but at a price. With this in mind attempts have been made to expose animals to attenuated parasites which cause less pathology but still allow protective immunity to develop. Attenuated parasites are also easy to produce (by irradiation), cost less than conventional anthelminthics and need at most 2 doses to provide very high levels of protection (Urquhart 1977). They are therefore the most attractive agent for use in vaccination and indeed have been the most successful to date, with one in particular proving to be a major commercial success. 'Dictol' the live irradiated vaccine against lungworm of calves, Dictyocaulus viviparus, did not, as was expected, herald a new era when vaccines would be produced against all the major helminth parasites of man and his domestic stock. Attempts to produce other irradiated vaccines have had disappointing results, not always related to the quality of the vaccine. The vaccines against lungworm of sheep, and hookworm of dogs are examples of vaccines that were unsuccessful because of lack of consumer interest and mismanagement in application respectively (see Urquhart 1977, Miller 1971, 1978). Recent reports of irradiated vaccines against bovine and ovine Schistosomes (Taylor et al 1976 Taylor et al 1978) suggest that the veterinary use of this method of

immunization is yet to be fully realised.

Another method of vaccination is that of heterologous immunization. Again this makes use of a live parasite, usually a related species, which causes less damage to the host or against which the host readily develops immunity. The object is to expose the host to some of the antigens shared between the parasites which may then prove to be protective. Extensive work has been done on cross immunity between Schistosome species (summarised by Eveland, Hsu and Hsu 1969) but whether there is cross-immunity between S. haematobium and S. mansoni in man remains unanswered. However, Nelson (1966) did find that the prevalence of S. bovis in cattle minimised S. haematobium infections in man. Systems involving heterologous protection have been used mainly as laboratory models and have not been employed in clinical trials.

There are also a number of recorded cases where immunity against helminths has been achieved by the use of agents which stimulate resistance non-specifically. Bacillus-Calmette-Guerin (BCG), has been found to be effective in protection against Echinococcus multilocularis in cotton rats (Rau and Tanner 1975) and against E. granulosis in jirds (Thompson 1976) by enhancing a pre-existing weak immune response. Obviously this non-specific stimulation of protection has the advantage that the host need not be exposed to the parasite or even to parasite antigens but has the disadvantage that the effectiveness of the technique may depend on the time of administration of the stimulant in relation to parasite exposure.

One of the major problems with research on parasitic diseases in man and in many animals is that only epidemiological and clinical studies are possible. These provide information on the current status

of the infection but cannot provide details of the previous exposure record of the host to the parasite. Only occasionally can the relative luxury of controlled experimental programmes of vaccination be afforded, and these in animals, not in man. The mechanisms which eliminate, or in some cases fail to eliminate parasites are not well understood. For these reasons much of the research on immunity to intestinal parasitic infection comes from the study of laboratory model systems. Some of these models are listed here (see Table 1) together with a summary of the host responses thought to be involved in mediating resistance to the parasite concerned. To a large extent current concepts of immune responses against intestinal nematodes have developed from the substantial body of work carried out with Nippostrongylus brasiliensis over the past 40 years. The discussion in this section, which is not intended to be an exhaustive literature review, therefore will largely be limited to consideration of this species.

The most commonly used method of assessing the immune status of the host after infection with N. brasiliensis is to count the number of worms in the intestine. Laboratory hosts are capable of expelling intestinal nematodes from the gut, at different times depending on the host and parasite strains and the immune status of the host. This expulsion is known as 'spontaneous cure' and was first observed (for N. brasiliensis) in rats by Africa (1931), who noticed that faecal egg counts from rats dropped sharply during the second week of infection and also that worms were lost from the intestine over a period of several weeks. Analysis of the immune response has been undertaken using a variety of histological, serological and cellular techniques of which the most fruitful have been adoptive and passive transfer and the use of ablation of response components followed by selective

TABLE 1

PARASITE	HOST	TIME TO EXPULSION (DAYS)	THYMUS DEPENDENCY OF EXPULSION	TRANSFER OF RESPONSE		EXPULSION ASSOCIATED WITH		
				SERUM	CELLS	OTHER CELLS	REAGINIC ANTIBODY	INFLAMMATION
Nippostrongylus brasiliensis	rat	12 - 15	+[1]	+[9]	+[9,17,18,19]	basophils eosinophils goblet cells[18,27,28,29] mast cells	+[36]	+[39]
	mouse	8 - 12	+[2]	+[10]	+[10]	mast cells	±	±
Trichinella spiralis	rat	14 - 18	+[3]	+[11]	+[11,20]	basophils, eosinophils[28] mast cells	±	+[40]
	mouse	4 - 18	+[4]	+[12]	+[21,22]	mast cells[30]	+[37]	+[41,42]
Trichostrongylus colubriformis	guinea-pig	15 - 20	+[5]	+[13]	+[23]	eosinophils[31] basophils	+[38]	+[43]

/ continued.

TABLE 1 / continued.

PARASITE	HOST	TIME TO EXPULSION (DAYS)	THYMUS DEPENDENCY OF EXPULSION	TRANSFER OF RESPONSE		EXPULSION ASSOCIATED WITH		
				SERUM	CELLS	OTHER CELLS	REAGINIC ANTIBODY	INFLAMMATION
Trichuris muris	mouse	11 - 14	+[6]	+[14]	+[14]	polymorphs[32]	-	-
Strongyloides ratti	rat	25 - 30	+[7]	+[15]	+[24]	mast cells[15,33] eosinophils	?	+[44]
Nematospiroides dubius	mouse	240+	+[8]	+[16]	+[25,26]	macrophages[34,35] eosinophils	?	?

1 Rose, Ogilvie, Hesketh and Festing (1979)

2 Jacobsen and Reed (1974)

3 Gore, Burger and Sadun (1970)

4 Ruitenberg, Elgersma, Kruizinga and Leenstra (1977)

5 Dineen and Adams (1971)

6 Wakelin and Selby (1974)

7 Moqbel (personal communication)

8 Prowse, Mitchell, Ey and Jenkin (1978)

9 Ogilvie and Jones (1968)

10 Love (1975)

11 Love, Ogilvie and McLaren (1976)

12 Denham (1969)

13 Connan (1972)

14 Selby and Wakelin (1973)

15 Moqbel (PhD Univ. London)

16 Behnke and Parish (1979)

17 Nawa and Miller (1978)

18 Nawa and Miller (1979)

19 Nawa, Parish and Miller (1978)

20 Despommier, McGregor, Crum and Carter (1977)

21 Wakelin and Lloyd (1976)

22 Wakelin and Wilson (1977)

23 Dineen and Wagland (1966)

24 Moqbel (in press)

25 Cypess (1970)

26 Behnke and Parish (in press)

27 Miller and Nawa (1979)

28 Ogilvie, Askenase, Rose (1980)

29 Befus and Bienenstock (1979)

30 Alizadeh and Wakelin (1980)

31 Rothwell and Dineen (1972)

32 Jenkins (PhD Univ. Glasgow)

33 Moqbel (1980)

34 Jones and Rubin (1974)

35 Chaicumpa, Jenkin and Fischer (1977)

36 Wilson and Bloch (1968)

37 Gabriel and Justus (1979)

38 Rothwell and Huxtable (1976)

39 Urquhart, Mulligan, Eadie and Jennings (1965)

40 Castro, Hessel and Whalen (1980)

41 Larsh (1975)

42 Wakelin and Wilson (1979)

43 Dineen, Gregg, Windon, Donald and Kelly (1977)

44 Moqbel and Wakelin (1979)

reconstitution. From this work has come a picture of the various factors known to be involved in mounting protective immune responses.

The idea that resistance to many infectious diseases was mediated by humoral factors rather than cellular components was in vogue in the thirties when the first attempts at passive transfer of immunity against N. brasiliensis were made. The role of serum factors, and more specifically antibodies, has been controversial. Sarles and Taliaferro (1936) passively protected rats against N. muris with serum from immune animals and there have been several subsequent records of successful passive transfer against this and other species (see Table 1). However the results are often inconsistent. This is not to say that serum is unimportant in immunity to nematodes, it may well play a key role, but at present this is not clearly defined. Indeed the findings of Jacobsen, Reed and Manning (1977) that N. brasiliensis may be expelled from mice lacking antibody production potential, suggests that in certain systems, at least, other factors are more important in causing worm expulsion.

Following on from their early work on the passive transfer of immunity to N. brasiliensis Taliaferro and Sarles (1939) examined the origin behaviour and function of cells involved in the inflammatory reactions to N. muris in rats. This was a histological rather than an immunological investigation. It was many years later, following the discovery of the role of the lymphocyte in immune responses, before any attempt was made to transfer immunity to nematodes by using cells. One of the first well-defined and successful reports was that of Dineen and Wagland (1966) working with T. colubriformis in isogenic guinea-pigs, who transferred immunity using cells obtained from the mesenteric lymph node (MLNC). This node is on the main route of lymphocyte traffic, by which cells from the intestine pass into the

thoracic duct lymph and re-enter the gut mucosa via the blood stream. It is an obvious source of cells capable of transferring immunity to enteral infections. Many successful cell transfers using mesenteric lymph node cells or thoracic duct lymph (TDL) cells (see Table 1) have now been recorded.

As immunologists have separated the lymphoid cells into their various subsets so parasitologists have attempted to transfer immunity with these defined cell populations. The major division into T- (thymus derived) -cells and B- (bursa[bone marrow] derived) -cells is the one that has been commonly employed in such work. Evidence is available from a number of systems indicating that the relative importance of these two cell types may be different in different systems. Wakelin and Wilson (1979) demonstrated that an immunoglobulin (Ig^-), T cell fraction obtained from the mesenteric lymph node was more efficient than an immunoglobulin positive, B cell fraction at transferring immunity to T. spiralis in mice. The reverse was found to be the case in rats using cells from TDL (Despommier, MacGregor, Crum and Carter 1977). In neither case however, were the T and B fractions pure, but merely enriched for the respective components.

The picture is further complicated by the inability to transfer immunity to N. brasiliensis in lethally irradiated rats and T. spiralis in lethally irradiated mice using MLNC alone as demonstrated by Dineen and Kelly (1973), Kelly, Dineen and Wagland (1973) and by Wakelin and Wilson (1980). These authors were able to transfer immunity only with immune MLNC plus bone marrow (myeloid, B.M.) cells. Furthermore it was shown that a period of BM differentiation was required before cell transfer was effective and it was concluded that the time delay reflects the period during which various other cell populations, macrophages, eosinophils, basophils are replaced.

Wakelin and Selby (1976) were able to transfer immunity to T. muris in mice with immune MLNC alone, so that accessory cell populations appear not to be necessary in all systems. Ogilvie, Love, Jarra and Brown (1977) showed that antibody-damaged worms could be expelled from irradiated rats, also without the aid of an accessory cell population.

There has been some speculation about the role of other non-lymphoid cell populations in worm expulsion. Non-lymphoid cell types are closely associated with inflammatory responses and, as the expulsion of many intestinal nematodes occurs when the gut is inflamed, it is hardly surprising that a T-cell mediated, non-specific inflammatory response has been proposed as the effective mechanism (Larsh and Race 1975). It is interesting to note that the expulsion of T. muris from mice is not associated with inflammation (see Table 1) and, as was mentioned earlier, does not appear to require accessory cell populations for effective transfer.

Other components of the inflammatory response have also been investigated. The role of amines, mast cells and IgE in worm expulsion received a great deal of attention in the sixties and early seventies. Three different mechanisms were prooosed:

1. the release of biogenic amines causing permeability changes in the intestinal mucosa and alteration of the gut environment to the detriment of the parasite. (Urquhart, Mulligan, Eadie and Jennings (1965).

11. increased gut permeability allowing antiworm antibodies to reach the worms (Barth, Jarrett and Urquhart 1966)

111. release of biogenic amines affecting worms directly. (Jones and Ogilvie 1971).

With T. colubriformis in guinea pigs amines appear to be directly involved in worm expulsion. When 5-hydroxytryptamine (5-HT), an amine present in basophils, was given intra-intestinally to guinea pigs early in T. colubriformis infection, worm expulsion was accelerated (Rothwell, Dineen and Love 1971); administration of amine inhibitors had the opposite effect. In other systems conflicting evidence and a failure to correlate the numbers of mast cells with worm expulsion has caused the hypothesis to be neglected.

The search has continued for the various components involved in worm expulsion and for this reason agents which have effects on inflammation and gut motility have been examined. One of these, Prostaglandin E_1 (PGE_1), was shown to affect N. brasiliensis worms in vitro (Richards, Bryant, Kelly, Windon and Dineen1977) and brought about worm expulsion in vivo (Dineen, Kelly, Goodrich and Smith 1974a). However recent evidence has failed to support the involvement of PGE_1 in the immune expulsion of N. brasiliensis from rats (Kassai, Redl, Jecsai, Balla and Harangozo 1980).

Other changes associated with inflammatory processes occurring during the expulsion of intestinal nematodes, such as increased levels of peroxidase enzymes, (a good measure of inflammation), villus atrophy and crypt hyperplasia,increased goblet cell numbers, intestinal transit times and fluid balance have all been examined but it has not been possible to link worm expulsion directly to any one of these (Castro, Roy and Stockstill 1974, Manson-Smith, Bruce and Parrott 1980, Miller 1979, Castro, Hessel and Whalen 1980 and Castro, Badial-Aceves, Smith, Dudrick and Weisbrodt 1976).

Recent reports have described the involvement of eosinophils and basophils in anti-worm activity in vitro and in vivo (RSTMH 1980) and

there has also been a revival of interest in the mast cell but the evidence for the involvement of this cell in the expulsion mechanism remains circumstantial (MacDonald, Murray and Fergùson 1980). As yet an all encompassing mechanism of worm expulsion has not been constructed, and it is indeed questionable if one is possible. There have been several attempts to link various components together in such a way as to explain worm expulsion in terms of antibody action on the worms plus a cellular involvement which effects expulsion (Ogilvie and Jones 1971). However, since the possibility exists that some or all of these components act independently and interdependently at different times and at different stages during infection in any single system, most workers avoid referring to any given mechanism as being the most important in worm expulsion.

THE PRESENT STUDY

One aproach which might provide useful information regarding the role of the immune system in regulating parasitic infection is the study of models in which expulsion (spontaneous cure) fails to occur; the one chosen for study here is *Nematospiroides dubius* in the mouse. *N. dubius* can survive in the intestine of the host for up to eight months (Ehrenford 1954). Coupled with this long survival is the failure of a primary infection to stimulate protective immunity to re-infection. Both these characteristics make it an ideal model for the study of chronic intestinal infections.

The species *N. dubius*, which belongs to the Heligmosomidae,was first described from the woodmouse *Apodemus sylvaticus* by Baylis (1926). Since then it has been recorded from a number of hosts ranging from the feral house mouse, *Mus musculus* to the salt marsh harvest mouse, *Reithrodontomys megalotis* (Forrester 1971) and the deermouse,

Peromyscus maniculatus (Babero and Matthias 1967). The rat has never been reported as a normal definitive host in N. America and Cross (1960) found it impossible to establish infections in rats without the use of immunosuporessant treatment. However, Skrjabin (1954) has reported natural infections in rats in Western Europe and in the U.S.S.R. It is possible that these discrepancies reflect the occurrence of strain differences and parasite adaptation and these topics will be discussed in a later section.

The life cycle of N. dubius is given below.

INFECTIVE (L_3) LARVAE
EXSHEATH IN STOMACH

ORAL INGESTION

EGGS HATCH
L_1 MOULT TO L_2,
L_2 MOULT TO L_3

PASS TO SMALL INTESTINE
AND PENETRATE MUCOSA
MOVE TO MUSCULARIS EXTERNA

WORMS MATURE, MATE,
EGG PRODUCTION BY
DAYS 9-10

JUVENILE PARASITES
EMERGE FROM MUSCULARIS
EXTERNA INTO LUMEN DAY 8
POST INFECTION

The details of the life cycle have been described by several authors. Their findings together with those obtained in this laboratory are shown in Table 2. Some of these results are controversial particularly those of Ehrenford (1954) who described only three moults when it is generally accepted that all nematodes have four. Experience has shown that with only minor modifications the life cycle described by Bryant (1973) is similar to the cycle obtained in this laboratory. The major differences in our system were

a) Most eggs had hatched by 30 hours.

TABLE 2: SUMMARY OF LIFE CYCLE OF N. DUBIUS

	FREE LIVING STAGES				PARASITIC STAGES			
	CULTURE TEMPERATURE	TIME OF HATCHING (HR)	TIME OF MOULTING (HR) L1 - L2	L2 - L3	TIME OF MOULTING AFTER INFECTION COMPLETE L2 - L3	L3 - L4 (L2 - L3)	L4 - JUV (L3 - L4)	(L4 - JUV)
SPURLOCK 1943	?	Infective larvae 4-6 days after start of culture			Two parasitic moults occurred			
EHRENFORD 1954	23 - 28 C	26	74+		Upon ingestion	(48 - 96)	(144 - 192)	
FAHMY 1956	22 C 26 C	23 - 24 19½ - 20	- 44	- 68 - 76+	- -	- -	- -	
DOBSON 1960	22 - 24 C	?	?+		24	(48)	(96)	(Before) (144)
BRYANT 1973	20 C	36 - 37	64 - 65	82 - 83+	18	90 - 96	144 - 166	
HAGAN ++	23 C	24 - 30	60 - 66	78 - 84+	Upon ingestion	72 - 96	144 - 178	

\+ preparasitic half moult

++ larvae stored 4 C prior to infection for parasitic stages

b) Pre parasitic moult was completed within minutes of ingestion.

c) No larvae, sheathed or exsheathed could be found free in the lumen of the stomach or intestine 24 hours after infection.

The pathology of N. dubius infection has been adequately described by Liu (1965) and will not be discussed here.

The main theme of the work presented in this thesis is the elucidation of the mechanism(s) by which N. dubius prevents or circumvents the immune response of the mouse. In analysing the mechanisms four main areas of study were defined

1. Chronicity of infection

To gain information on the immunosuppressive effect and on the long term survival of N. dubius, the effect of N. dubius on the ability of mice to respond to concurrent T. spiralis infection was examined.

2. Interference with cellular function

The key cells involved in immune responses are lymphocytes and macrophages if these are interfered with then immune responses may be altered. For this reason the in vivo and in vitro responsiveness of mesenteric lymph node cells and macrophages during N. dubius infection were examined.

3. Stimulation of immunity to Reinfection

Before examining the role of cells and serum in immunity to N. dubius it was essential to have methods of immunization which stimulated protection in donor

animals. Various immunization schedules were investigated and cross immunity studies with field strain of *N. dubius* were undertaken. The effect of splenectomy on the course of *N. dubius* infection is reported.

4. Use of Attenuated larvae in immunization

Up until now the stimulation of immunity to *N. dubius* has involved the use of multiple immunizing schedules. Irradiated larvae were used in an attempt to shorten these lengthy schedules. The effect of cobalt 60 irradiation on the parasite is also described.

GENERAL MATERIALS AND METHODS

ANIMALS

a) MICE

The following strains of mice were used:- inbred NIH, inbred C57 BL/10 and outbred CFLP, purchased from Anglia Laboratory Animals, Huntingdon or from Bantin and Kingman, Grimston, and helminth free inbred DBA_1, F_1NIH x C57 BL/10 and outbred CFLP bred in this laboratory.

In most experiments mice were caged in groups of six in polypropylene cages 48cm x 15cm x 13cm (North Kent Plastics Ltd.). Sawdust or wood shavings was provided as bedding and replaced twice per week. Animal rooms were maintained on a 12 hour light-dark cycle in winter but followed day length in summer. Tap water and pelleted food (Grain Harvesters Ltd.) was available to the animals ad libitum.

Field mice (Apodemus sylvaticus) were trapped, overnight within the Garscube Estate, Bearsden, Glasgow by means of baited Longworth traps. On return to the laboratory these mice were sexed and caged singly in metal cages.

b) RABBITS

Three kg. New Zealand White rabbits were used for the production of antisera. These were housed singly in slatted cages in the animal house of the Department of Microbiology, University of Glasgow.

c) PARASITES

Nematospiroides dubius

The strain used was obtained in 1975 from the Wellcome Research Laboratories, Beckenham, and has since been maintained in mice, usually outbred CFLP. Infective third-stage larvae (L_3) were obtained by culture of faecal material from infected stock

mice in the following manner:-

stock mice were placed in a cage with a metal grid base, suspended above a plastic tray containing tap water. After four or five hours the faecal pellets were collected, washed twice in distilled water and ground to a paste. About 0.25 - 0.5g amounts of this paste were spread centrally on 7cm filter papers (Whatmans No. 1) which were then placed in petri dishes containing 1ml of distilled water. The dishes were placed in a dark cupboard at room temperature (23°C) for 6-8 days. During this time the filter paper was examined every two days and kept moist with distilled water. This procedure allows the eggs of N. dubius to hatch and develop into infective larvae (L_3), which then migrate into the water around the paper.

After collection, gross faecal contaminants were removed by passing the larval suspension through several layers of nylon gauze. Larvae were centrifuged at 300g for 5 minutes and resuspended in distilled water to give a count of 1000-2000/ml. This stock suspension was held at 4°C in a sealed 50ml glass conical flask. Larvae remain viable (infective) for at least three months under these conditions.

Mice were infected orally with the required number of larvae in 0.2ml distilled water using a syringe and a blunt 21 gauge needle. Care was taken to avoid cross contamination of laboratory strain cultures and those of the field strain of N. dubius collected from Apodemus sylvaticus. The number of larvae/dose was found to be accurate to within $\pm$ 10%.

Trichinella spiralis

The strain used in the experiments was obtained from the London School of Hygiene and Tropical Medicine and maintained by passage through outbred CFLP mice. Infective larvae were obtained by digestion of stock mice which had been infected for at least sixty days. One stock mouse was killed, eviscerated, minced and digested for 2-3 hours in 500ml of 0.5% pepsin in 0.5% HCl at 37°C with continuous agitation. After removal of the coarse sediment by filtration through gauze the larvae were collected by repeated washing and sedimentation in 0.9% NaCl. The larvae were suspended in 0.2% agar and the total volume adjusted to give the required dose in 0.2ml.

ADULT WORM RECOVERY

Both N. dubius and T. spiralis were recovered using a modified Baermann technique (Wakelin and Lloyd 1976). Mice were killed with chloroform and the entire small intestine was removed and cut open along its length. It was then placed on a piece of gauze and submerged in 40ml of pre-heated (40°C) Hanks' balanced salt solution (HBSS) in a 50ml beaker. Incubation was continued for 4-5 hours during which time worms migrated through the gauze. At the end of the incubation period the intestine was removed from the beaker. When recovering N. dubius it was found that raising the temperature to 45-50°C for 30 minutes after removal of the intestine from the beaker prevented the worms from becoming entangled with one another.

Worms were counted in a petri-dish soon after incubation. If not counted immediately beakers were stored overnight at 4°C.

EGG COUNTS

Daily samples of 0.5-1.0g of fresh faeces were collected in a plastic bucket from each group of mice between 9-10 am. The faeces were ground to a paste in 30ml of a solution of saturated salt and tap water (1:1) and passed through a metal sieve of mesh size 300 microns. The coarse faecal matter was further washed with 30ml of a saturated salt solution.

At least four samples of the suspension were counted in a modified McMaster chamber as described by Gordon and Whitlock (1939). Egg counts are given as eggs per gram of faeces.

ANTHELMINTHIC

Strongid-P Paste (Pyrantel embonate, Pfizer Ltd.) was diluted with distilled water and used at a dose of 100mg/kg to remove N. dubius adult worms; such a dose is 100% effective within one day. No residual effect on infections given one day later was ever recorded.

IMMUNOSUPPRESSION

Cortisone acetate (Cortistab, The Boots Company Ltd.) given subcutaneously was used at a dose of 1.0-1.25mg/mouse every two days.

ANTIBIOTIC

Oxytetracycline hydrochloride (Terramycin, Pfizer Ltd.) at a concentration of 165mg/l was given in the drinking water to mice which had undergone surgery or immunosuppression as well as to their relevant controls.

SERUM COLLECTION

Blood was collected from mice by cardiac puncture, using a 19 gauge needle and a 2.0ml syringe. The blood was allowed to clot in a glass centrifuge tube for 3 hours at room temperature and then kept

at 4°C overnight. The tube was centrifuged for 10 minutes at 1800g and the serum was removed and stored in small aliquots at either -20°C or -40°C. When required, serum was injected into recipient mice intraperitoneally (i/p). Doses are given in each experiment.

PRESENTATION AND STATISTICAL ANALYSIS OF RESULTS

Almost all the results in this thesis are presented in the form of bar charts and line graphs rather than in the form of tables. It is hoped that this will ease their interpretation. Unless otherwise stated error bars indicate $\pm$ one standard deviation about the mean. Statistical analyses are not given when results are obviously different or when no differences exist. The statistical test used was the non-parametric Wilcoxon test (Sokal and Rohlf 1969). A value of $P<0.05$ was considered to be significant.

SOLUTIONS

Modified Hanks' Balanced Salt Solution (MHSS) (Hopkins and Stallard 1974).

Hanks' solution was modified by excluding glucose and $NaHCO_3$ and increasing the remaining salts to an osmotic pressure of 300 m-osmole.

SOLUTION 1

NaCl	168g
KCl	8g
KH_2PO_4	2g
Na_2HPO_4	4g
0.2% Phenol Red	200 ml

Made up to 2 litres with deionized water.

SOLUTION 2

$CaCl_2.2H_2O$	3.92g
$MgCl_2.6H_2O$	2.00g

Made up to 2 litres with deionized water.

105ml of solutions 1 and 2 were mixed and made up to 1 litre with deionized water. (Final pH 7.2).

Phosphate buffered saline (pH 7.2).

Na_2HPO_4	2.84g
$NaH_2PO_4.2H_2O$	2.76g
NaCl	9.00g

Make up to 1 litre.

Trypan Blue (cell viability)

Stock solution 2g trypan blue (BDH) made up to 100ml with deionized water. Diluted 1:20 in HBSS for use in dye exclusion test.

Weigert's Iron Haematoxylin (Lille modification 1968)

SOLUTION A

Ferric chloride	($FeCl_3 \cdot 6H_2O$)	2.5g
Ferrous sulphate	($FeSO_4 \cdot 7H_2O$)	4.5g
Hydrochloric acid		2.0ml
Distilled water		298.0ml

SOLUTION B

Haematocylin	1.0g
95% ethyl alcohol	100.0 ml

Mix solutions A and B. Solution turns black at once, can be used 2-3 weeks or until it turns brown. (See Humason 1972).

Effect of *N. dubius*

on the ability of mice

to respond to

T. spiralis infection.

SECTION 1(1)

INTRODUCTION

The functional interaction between a parasite and the immune responses of the host is complex, involving an array of factors. In any relationship the relative contribution of each of these factors is constantly changing and so a number of possible outcomes exist. At one extreme parasites may stimulate genetically determined host responses which result in the eventual elimination of the infection and confer resistance to subsequent re-infection, as is the case with most of the parasites listed in Table 1 (see General Introduction). At the other extreme parasites may survive by evasion or impairment of the host responses. Evasion is best characterized by the Trypanosomes which change the antigenic structure of their surface glycoprotein coat, by the Schistosomes which acquire host blood group antigens onto their surface and by the Taeniid Cestodes e.g. T. taeniaeformis, which produce anti-Complement factors. Impairment of host responses is also associated with Trypanosomes which initiate a non-specific polyclonal B-cell activation (Hudson, Byner, Freeman and Terry 1976). A number of mechanisms have been proposed to explain depression of host responses by helminth parasites e.g. prevention of lymphocyte proliferation by worm factors (Dessaint, Camus, Fischer and Capron 1977) and active cytotoxicity of worm ES products for rodent lymphoid cells (Goose 1975, Faubert and Tanner 1975). No convincing evidence exists to show that such factors are responsible for the failure of the immune system to eliminate the parasites which produce them. Another suggestion is that antibodies produced against worm products complex with antigens before they can stimulate the mechanisms which would normally lead to parasite elimination. Infections with the nematode N. dubius are associated with a marked

immunodepression and suppress responses to a wide variety of heterologous antigens as shown in Table 3, (overleaf).

The mechanisms underlying the suppression are not well understood. Brown, Crandall and Crandall (1976) have suggested that suppression is due to an increase in the catabolic rates of all immunoglobulin G subclasses, which occurs as a result of the parasite induced IgG_1 hypergammaglobulinaemia. However their hypothesis assumes normal levels of IgG synthesis which are unlikely to occur during infection. Bartlett and Ball (1974) considered that suppression might be caused by blocking antibodies which complexed with antigen had a chance to stimulate a response. It is conceivable that the increased immunoglobulin levels in infected mice would also have this effect, resulting in a generalised and non-specific depression of responses to heterologous antigens.

The possibility that parasite induced suppression of responses to heterologous antigens occurs as a result of 'antigenic competition' or 'antigen induced suppression' (AIS) must be considered. AIS occurs when two antigens are administered sequentially but the mechanisms underlying the failure to respond to the second antigen are not fully understood (Pross and Eidinger 1974). AIS may have some part to play in parasite induced suppression (PIS). During nematode infections the host is exposed to a wide variety of parasite antigens, produced at different stages of infection, only some of which are important in terms of host protective immune responses. The probability of AIS occurring between two antigens from one parasite or between antigens from unrelated parasites must be relatively high. Of course this has important implications for vaccination programmes in endemic areas. For example, of the cases of failure of vaccination

TABLE 3

ANTIGEN	ROUTE OF ADMINISTRATION	RESPONSE MEASURED	REFERENCE
Influenza virus	nasal	Virus titre	Chowaniec, Wescott and Congdon (1972)
N. brasiliensis	oral	Faecal egg counts, worm recovery	Colwell and Wescott (1973) Jenkins (1975)
SRBC	oral	Haemagglutination (anti-SRBC)	Shimp, Crandall, Crandall (1975)
T. muris	oral	Worm recovery	Jenkins and Behnke (1977)
T. spiralis	oral	Worm recovery	Behnke, Wakelin and Wilson (1978)

using 'Dictol' many were attributed to the effects of concurrent gastrointestinal parasitism (Peacock and Poynter 1979). Analysis of PIS is undoubtedly complex, even more complex than analysis of classical AIS using two sequentially administered, chemically defined antigens. However, it is known that N. dubius interferes with the immune response to T. spiralis and T. muris (Jenkins et al 1977, Behnke et al 1978), and it seemed useful to study the suppressive effect of N. dubius in terms of the mechanisms known to be involved in protection against T. spiralis. This work is described in section 1(1) and is followed by a section describing lymphocyte migration patterns in mice infected with N. dubius and T. spiralis. Lymphoblasts originating in the gut-associated lymphoid tissue (GALT) are known to home to the gut, suggesting that homing patterns may have an important influence on the responses which stimulate immune (re-)activity via the GALT. Increased levels of cell homing have been recorded in at least three systems involving nematode parasites (Table 4).

Manson-Smith, Bruce and Parrott (1979) demonstrated that the expulsion of T. spiralis is preceded by enhanced lymphoblast localization in the intestine and also by partial villus atrophy and crypt hyperplasia, but there has been no direct link between any of these events and the expulsion process.

The exact cause of lymphoblast localization is unclear but it is known that it is not an antigen driven event, which may be associated with inflammatory processes (Asherson, Allwood and Mayhew 1973). The cells which home to the intestine may be either T-blasts or B-blasts. The latter with surface and intracellular IgA (suggesting IgA production), transform to IgA producing plasma cells on reaching the intestinal lamina propria (Guy-Grand, Griscelli and Vassalli

TABLE 4

NEMATODE	HOST	DAY OF INFECTION SHOWING ENHANCED LYMPHOBLAST LOCALISATION	
T. spiralis	Mice	2 + 4	Rose, Parrott and Bruce 1976a and b
T. spiralis	Rats	9 (Point Assay only)	Love and Ogilvie 1977
N. brasiliensis	Rats	9 + 12	Love and Ogilvie 1977

1974), in response to orally administered antigens. In view of the immunosuppressive effect of N. dubius on responses to orally administered antigens it seemed important to study the effect of infection on lymphoblast homing to the small intestine.

SECTION 1(1)

MATERIALS AND METHODS

PREPARATION AND TRANSFER OF CELL SUSPENSIONS

The mesenteric lymph node was stripped of connective tissue and fat *in situ* and then excised and placed in cold (4°C) Medium 199 (Gibco, Europe), with L-glutamine and Hepes buffer. This medium was supplemented with 10% foetal calf serum (FCS, Gibco, Europe) and 10i.u./ml heparin (B.D.H.). The low temperature caused the remaining fatty tissue to show up clearly and this was removed before the node was crushed through a nylon mesh sieve into a plastic petri dish using a rubber tipped syringe plunger. The petri dish was raised at one side and left to stand for 4-5 minutes. This allowed cell clumps and debris to sink to the bottom of the dish. The supernatant was collected and centrifuged at 200g. for 5 minutes and the cells were resuspended in a known volume of fresh medium. One drop of this suspension was mixed (1 in 20) with a 2% w/v solution of trypan blue in HBSS and the number of live (i.e. dye-excluding) cells counted in a haemacytometer. Viability was usually greater than 90%. The concentration of cells in the suspension was then adjusted to give the required number in 0.2-0.25ml. Cells were injected intravenously (i/v) into recipient mice via a lateral tail vein. Cell suspensions from spleens were prepared in a similar way. No treatment to remove erythrocytes was undertaken.

CELL LABELLING

Lymphocytes were prepared from the MLN and spleen as described above except that the medium used was RPMI 1640 (without glutamine or bicarbonate, Flow Laboratories) modified by the addition of 0.8ml of 7.5% sodium bicarbonate (Flow Laboratories), 1ml of 200mM

glutamine (Gibco, Europe), 10ml of heat inactivated FCS, 100 i.u. heparin, 1000 i.u. penicillin and 1000 g streptomycin per 100ml. Four ml aliquots of medium were placed in sterile plastic tissue culture grade tubes (100 x 16mm Nunclon Delta) and a further 1ml of medium containing 5 x 10^7 cells was added.

The tubes were gassed with 2% CO_2, and 5-[^{125}I] Iodo-2'-deoxyuridine (specific activity 185 G Bq/mg, Radiochemicals Centre, Amersham) in 0.9% NaCl was added to give a concentration of 1 Ci isotope to 10^7 cells/ml. The cells were incubated for two hours at 37 $\pm$ 0.5°C, centrifuged at 200g for 5 minutes at room temperature and, after removal of the supernatant, washed 3 times and resuspended in Medium 199 (without serum or heparin). Cells were counted, assessed for viability and 2-3 x 10^7 viable cells were injected (i/v) into recipient mice. An injected dose equivalent was retained for counting.

RECOVERY AND COUNTING OF LABELLED CELLS

Recipient mice were killed 24 hours after injection of labelled cells and the small intestine, MLN, spleen and liver were removed, rinsed in HBSS and placed in plastic vials for counting. The organs, together with the injected dose equivalent and two empty vials for background, were counted in a Packard Tricarb Liquid Scintillation Spectrometer. The results were expressed as % injected dose (% ID) or % Total recovered radioactivity (% TR) as shown below:

$$\% \text{ ID} = \frac{\text{ORGAN COUNT - BACKGROUND COUNT}}{\text{INJECTED DOSE COUNT - BACKGROUND COUNT}} \times \frac{100}{1}$$

and

$$\% \text{ TR} = \frac{\text{ORGAN COUNT - BACKGROUND COUNT}}{\text{TOTAL COUNT (ALL ORGANS) - (4 x BACKGROUND COUNT)}} \times \frac{100}{1}$$

'IN VIVO' LABELLING

In order to assess blast cell activity '<u>in vivo</u>' mice were injected with 2 Ci 5-[^{125}I] Iodo-2'-deoxyuridine in 0.9% NaCl (total volume 0.2ml) via a lateral tail vein. Two hours later these mice were killed and the MLN, spleen, axial lymph node, Peyers' patches (PP) and an equal number of intestinal snips (as PP) removed since dividing epithelial cells would also take up the label. These were washed in 70% alcohol for three days (1 change each day) to remove unbound label. They were then placed in plastic vials and counted in a Packard Tricarb Liquid Scintillation Spectrometer (see recovery and counting of labelled cells).

Following counting all the organs were dried in an oven (100 C) in a fume cupboard, usually overnight, and weighed. Results are expressed as total activity per tissue and as activity /mg tissue. Axial lymph node counts are not given as these were never above background levels.

SECTION 1(1)

RESULTS

TIME COURSE OF EXPULSION OF T. SPIRALIS DURING CONCURRENT N. DUBIUS INFECTION

As a preliminary to the experiments involving cell transfers an examination of the effect of a primary infection of N. dubius on a concurrent T. spiralis infection was undertaken. Two experiments were performed. In both of these 24, 6 week old NIH mice were infected with 300 N. dubius and 2-3 hours later these mice plus a group of previously uninfected, age matched controls (24), were given an infection of 300 T. spiralis. Mice from both groups were killed on days 8, 12, 16 and 20 post infection. The results are shown in Figures 1 (Experiment 1) and 2 (Experiment 2).

In both experiments there was an 8 day delay in the expulsion pattern of T. spiralis from mice concurrently infected with N. dubius when compared to that from the group infected with T. spiralis alone. This delay has been found to be a consistent effect when N. dubius is given at the levels used in these experiments. The decreased numbers of T. spiralis recovered on day 8 in experiment 1, (see Figure 1) may have been caused by the expulsion process starting slightly earlier than is usual i.e. day 7. From previous studies and from results obtained in these and in subsequent experiments there is no evidence to suggest that the level of establishment of T. spiralis is in any way affected by a pre-existing N. dubius infection. Similarly, at the levels of infection used in these experiments the N. dubius recovery remains as high as would be expected from a single infection in naive animals (see Figure 3), i.e. greater than 80% of the inoculum. At day 8 the recovery is low as

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FIGURE 1

Mean recovery (MWR $\pm$ SD) *T. spiralis* in single and double infection groups

- ● *T. spiralis* only
- ○ *T. spiralis* plus *N. dubius*

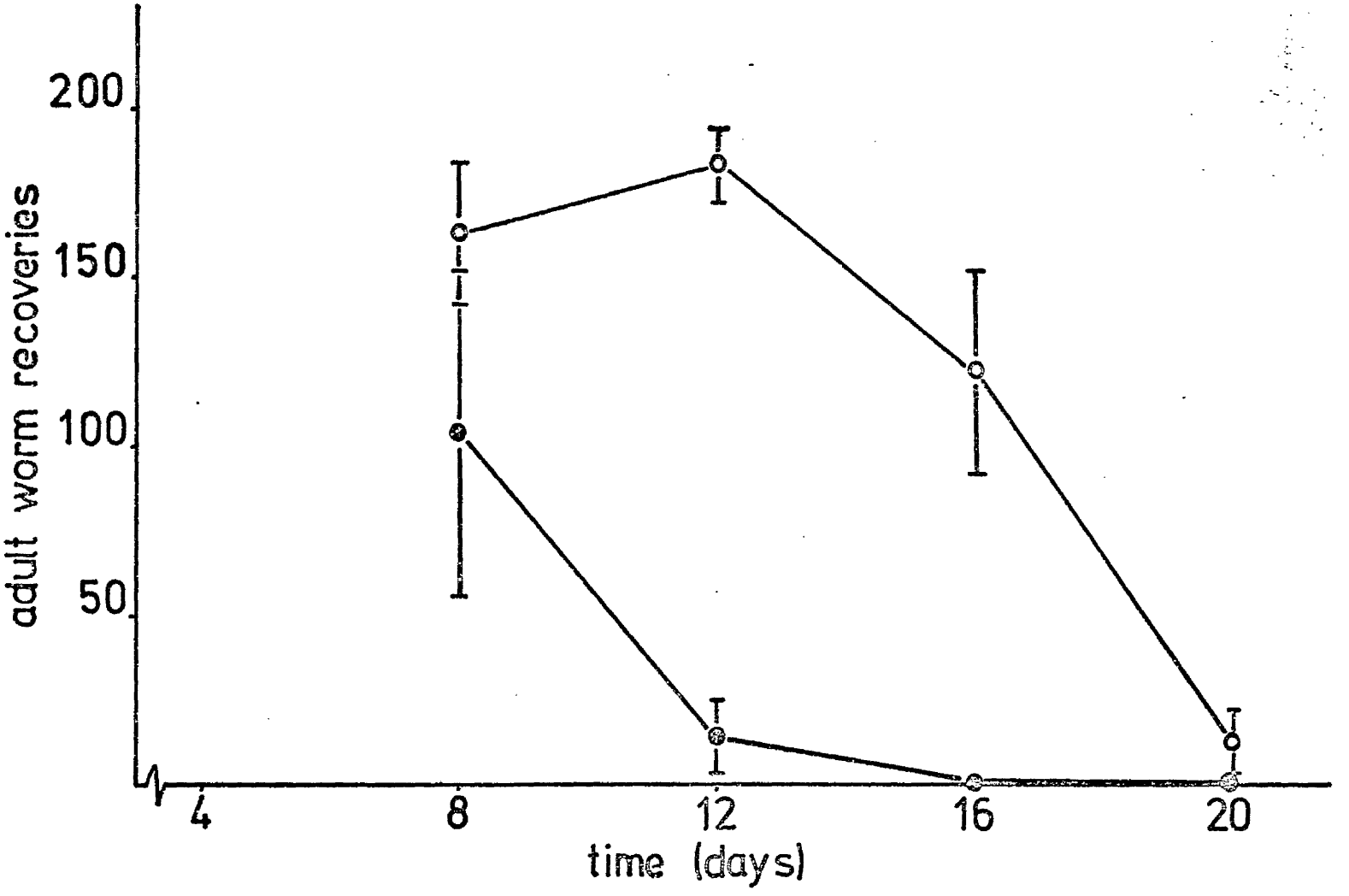
200
150
100
50
adult worm recoveries
4
8
12
16
20
time (days)

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FIGURE 2

Mean recovery (MWR $\pm$ SD) *T. spiralis* in single and double infection groups

- ● *T. spiralis* only
- ○ *T. spiralis* plus *N. dubius*

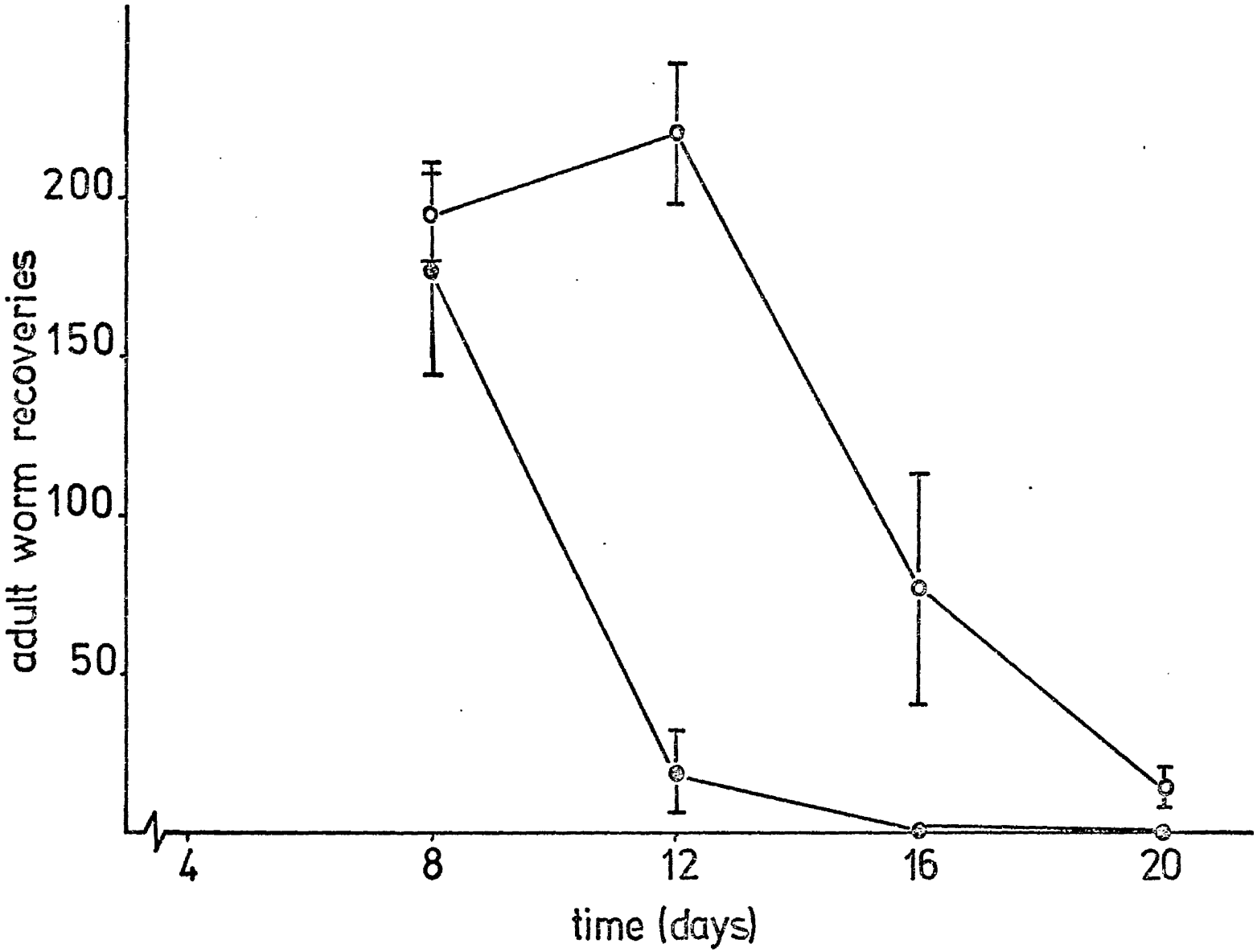
adult worm recoveries
200
150
100
50
4
8
12
16
20
time (days)

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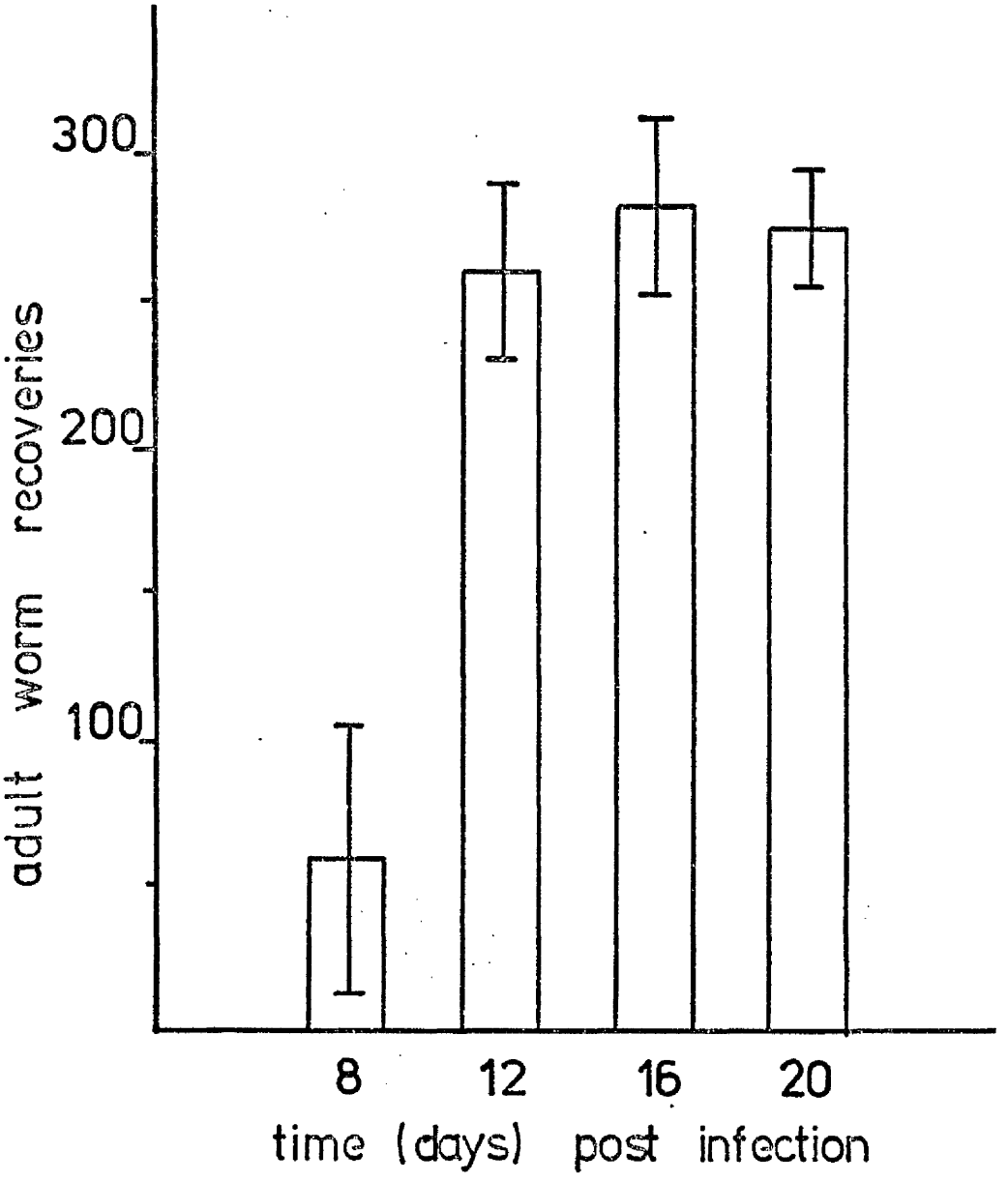
FIGURE 3

Mean recovery (MWR $\pm$ SD) *N. dubius* in mice concurrently infected with *T. spiralis*


300
200
100
adult worm recoveries
8
12
16
20
time (days) post infection

the worm has not yet emerged into the gut lumen.

TRANSFER OF IMMUNITY TO T. SPIRALIS USING CELLS FROM SINGLY AND CONCURRENTLY INFECTED DONORS

Twenty four, 8 week old NIH mice were infected with 370 T. spiralis, day 0. Twelve of these had been infected 2-3 hours earlier with 250 N. dubius. These mice were to serve as donors of cells for transfer on day 7.

On day 7 the MLN were removed from the single and double infection donors and single cell suspensions were prepared. The cells were transferred to recipients, 4×10^7 cells per recipient as shown in the schedule below.

DONOR		RECIPIENT	GROUP
Nd x Tsp	4×10^7 MLNC	+ Tsp	A
Tsp	4×10^7 MLNC	+ Tsp	B
	4×10^7 MLNC	+ Tsp + Nd	C
		Tsp only	D

As indicated, after cell transfer, mice were infected with 310 T. spiralis and/or 300 N. dubius and killed 8 days later. (Experiment 3, Figure 4).

MLNC taken from doubly infected donors on day 7 were not able to transfer immunity, (as assessed by adult worm recovery on day 8), to recipient mice. MLNC from mice infected with T. spiralis alone, and transferred to doubly infected recipients (Group C) also failed to transfer the faster expulsion characteristic of a successful MLNC transfer (Group B) (see Figure 4). However this failure to effect expulsion was not due to an inability of the cells to transfer immunity. In all groups given MLNC in this experiment the

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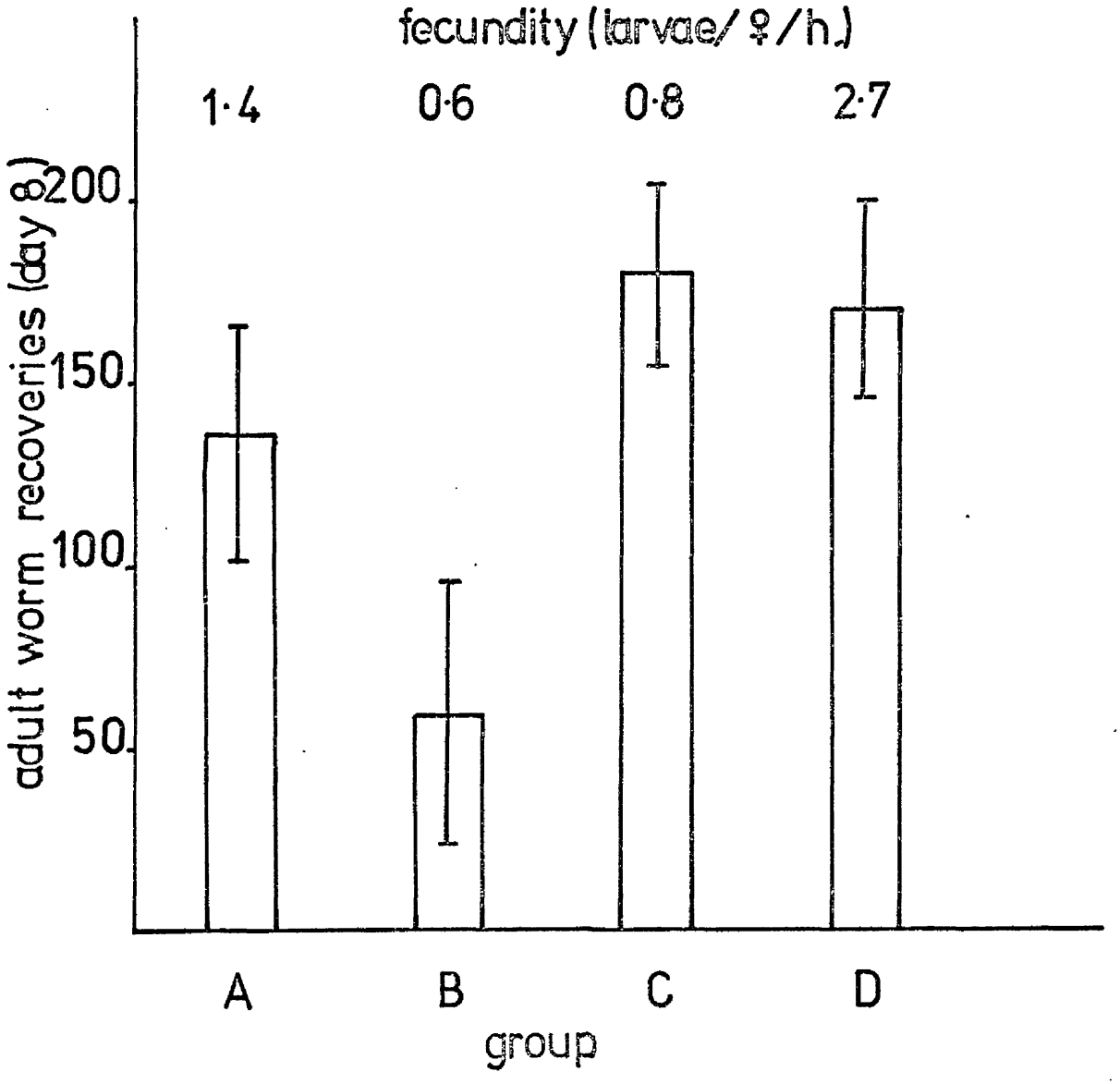
FIGURE 4

Mean recovery (MWR $\pm$ SD) T. spiralis on day 8 of infection after transfer of MLNC

DONOR		RECIPIENT	GROUP
Nd x Tsp	4×10^7 MLNC +	Tsp	A
Tsp	4×10^7 MLNC +	Tsp	B
	4×10^7 MLNC +	Tsp + Nd	C
		Tsp only	D

Fecundities larvae/female/hour are shown above each group.


fecundity (larvae/♀/h.)
1·4
0·6
0·8
2·7
adult worm recoveries (day 8)
200
150
100
50
A
B
C
D
group

fecundity of female T. spiralis was significantly reduced when compared to control day 8 values. Values for fecundities, larvae/female/hour are shown above the worm recovery charts in Figure 4. Exposure of the MLNC to an N. dubius infection in the donor or in the recipient slightly abrogated their effect on worm fecundity.

The MLNC donors in the above experiment also acted as SC donors and cells were transferred and recipients infected using a similar schedule, except that only 2.8×10^7 SC were transferred (Experiment 4, Figure 5).

DONOR		RECIPIENT	GROUP
Nd x Tsp	2.8×10^7 SC	+Tsp	A
Tsp	2.8×10^7 SC	+Tsp	B
	2.8×10^7 SC	+Tsp + Nd	C
		Tsp only	D

Recipient mice were infected with 310 T. spiralis and/or 300 N. dubius and killed on day 8 post transfer/infection. Spleen cells were ineffective in all transfers (see Figure 5). Neither worm numbers nor fecundity of female worms was affected even when the cells were taken from singly infected (T. spiralis) donors and transferred to recipients given only T. spiralis (Group B). There was no evidence for either delayed T. spiralis expulsion or higher establishment in SC recipients.

In a subsequent experiment (Experiment 5) only singly infected donors (T. spiralis) were used. In order to gain more information about the expulsion process, and to find out if the action of MLNC in N. dubius infected recipients, like worm expulsion was simply delayed. The experiment was extended to give data for day 8 and

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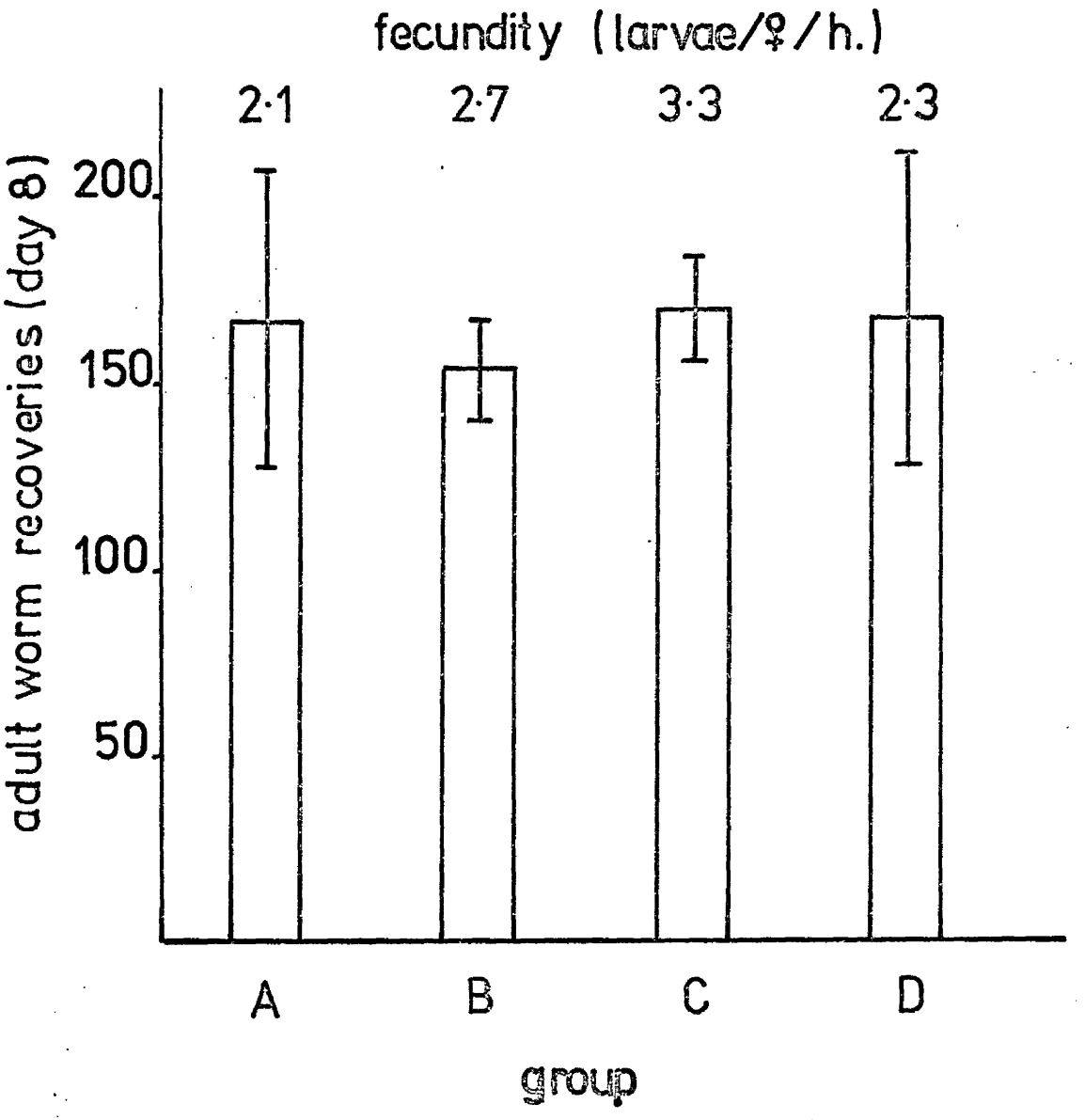
FIGURE 5

Mean recovery (MWR ± SD) *T. spiralis* on day 8 of infection after transfer of SC

DONOR		RECIPIENT	GROUP
Nd x Tsp	2.8×10^7 SC +	Tsp	A
Tsp	2.8×10^7 SC +	Tsp	B
	2.8×10^7 SC +	Tsp + Nd	C
		Tsp only	D


fecundity (larvae/♀/h.)
2·1
2·7
3·3
2·3
adult worm recoveries (day 8)
200
150
100
50
A
B
C
D
group

day 12 post transfer. Twelve 6-8 week old male NIH mice were used as MLNC donors, the cells being taken seven days after infection with 300 T. spiralis. The protocol is outlined below:

DONOR		RECIPIENT	GROUP
Tsp	3×10^7 MLNC	Tsp	A
	3×10^7 MLNC	Tsp +.Nd	B
		Tsp + Nd	C
		Tsp only	D

Cell recipients and respective controls were infected with 370 T. spiralis and/or 300 N. dubius. Mice were killed on days 8 and 12 post transfer (6/group). The results are shown in Figure 6.

The expulsion of T. spiralis from control mice followed the normal pattern found in NIH mice with large numbers of worms present on day 8 falling to low levels by day 12 (Group D). In the group given MLNC plus T. spiralis only (Group A), the cells have had the effect of reducing worm numbers, although with some variability. However there was no evidence for any loss of T. spiralis from the concurrent infection control group (Group C) and even more remarkable the MLNC had failed to operate in doubly infected recipients even up to day 12 (Group B) when mice which had not been given cells would normally have low worm burdens. Once again the transferred MLNC did have an effect on female worm fecundity. In both the groups given MLNC (Groups A and B) there was a significant reduction in worm fecundity on day 8 when compared to controls (Groups C and D). Values for fecundities are shown above worm recoveries in Figure 6.

The lengths of female worms were also recorded by measuring samples, usually 20, from each group using a camera lucida. The results are shown in Table 5 . No significant difference was

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FIGURE 6

Mean recovery (MWR $\pm$ SD) T. spiralis on days 8 and 12 of infection after transfer of MLNC

DONOR		RECIPIENT	GROUP
Tsp	3×10^7 MLNC +	Tsp	A
	3×10^7 MLNC +	Tsp + Nd	B
		Tsp + Nd	C
		Tsp only	D

Fecundities larvae/female/hour are shown above each group.

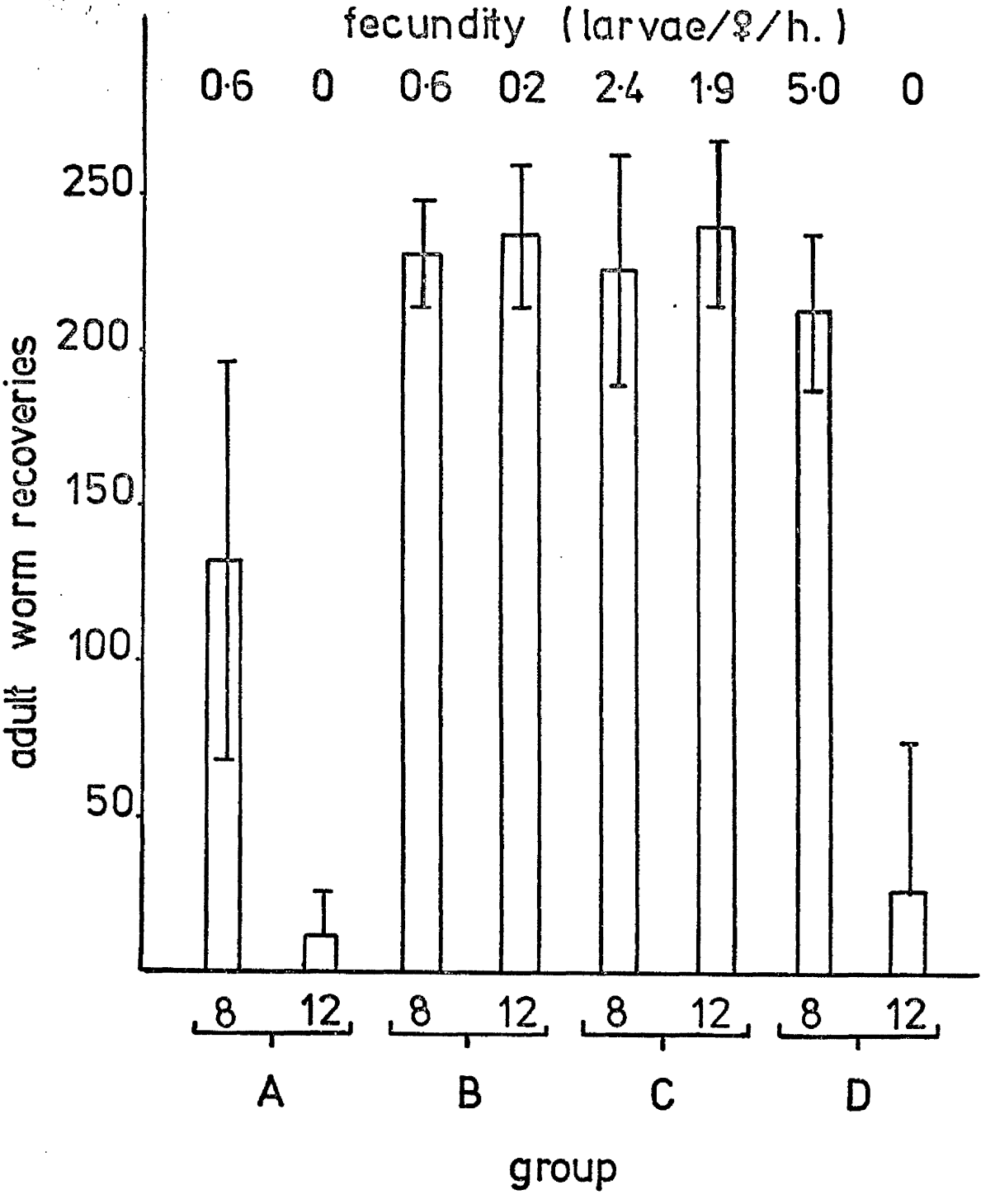
fecundity (larvae/♀/h.)
0·6
0
0·6
0·2
2·4
1·9
5·0
0
250
200
150
100
50
adult worm recoveries
8
12
8
12
8
12
8
12
A
B
C
D
group

TABLE 5

Mean lengths of adult female T. spiralis worms recovered from Groups A, B, C and D from experiment 5 (Figure 6)

GROUP	DAY 8	DAY 12
A	1.34 ± 0.16	1.58 ± 0.17
B	1.35 ± 0.22	2.19 ± 0.56
C	1.46 ± 0.23	1.85 ± 0.37
D	1.80 ± 0.29	1.40 ± 0.11

(Lengths in mm ± SD)

found between worm lengths from any of the groups on days 8 and 12.

This experiment was repeated with a similar protocol except that MLNC donor mice were infected with 270 T. spiralis. Cells were taken on day 7 and transferred to recipients (2×10^7 MLNC/recipient). Recipients were infected with 270 T. spiralis and/or 300 N. dubius and killed on days 8 and 12 post transfer as before. The results are shown in Figure 7 (Experiment 6). Once again mean adult worm recoveries in group D followed the pattern characteristic of NIH mice. Group A, given 2×10^7 MLNC and infected with T. spiralis only showed a significant reduction in worm burdens on day 8, although again there was some variability, and had no worms present on day 12. Both double infection groups (Groups B and C) had similar worm recoveries on days 8 and 12 with no evidence of worm expulsion in the group given MLNC (Group B). Female worm fecundities were reduced in groups A and B on day 8 when compared to groups C and D respectively. It was not possible to measure day 12 fecundities in groups A and D but the fecundity of group C had increased by day 12 showing a marked improvement on the day 8 figure. The low value on day 8 for group B can only be attributed to the presence/action of MLNC but why this depressed larval production was not maintained until day 12 is difficult to explain.

TRANSFER OF MLNC FROM DOUBLY INFECTED DONORS

In all previous experiments MLNC had been taken from singly or doubly infected donors on day 7 post infection. In the case of doubly infected mice the expulsion of T. spiralis is delayed until days 16-20 (see Figures 1 and 2). On the other hand single infection donors expel their worm burdens by days 8 to 12. In order to

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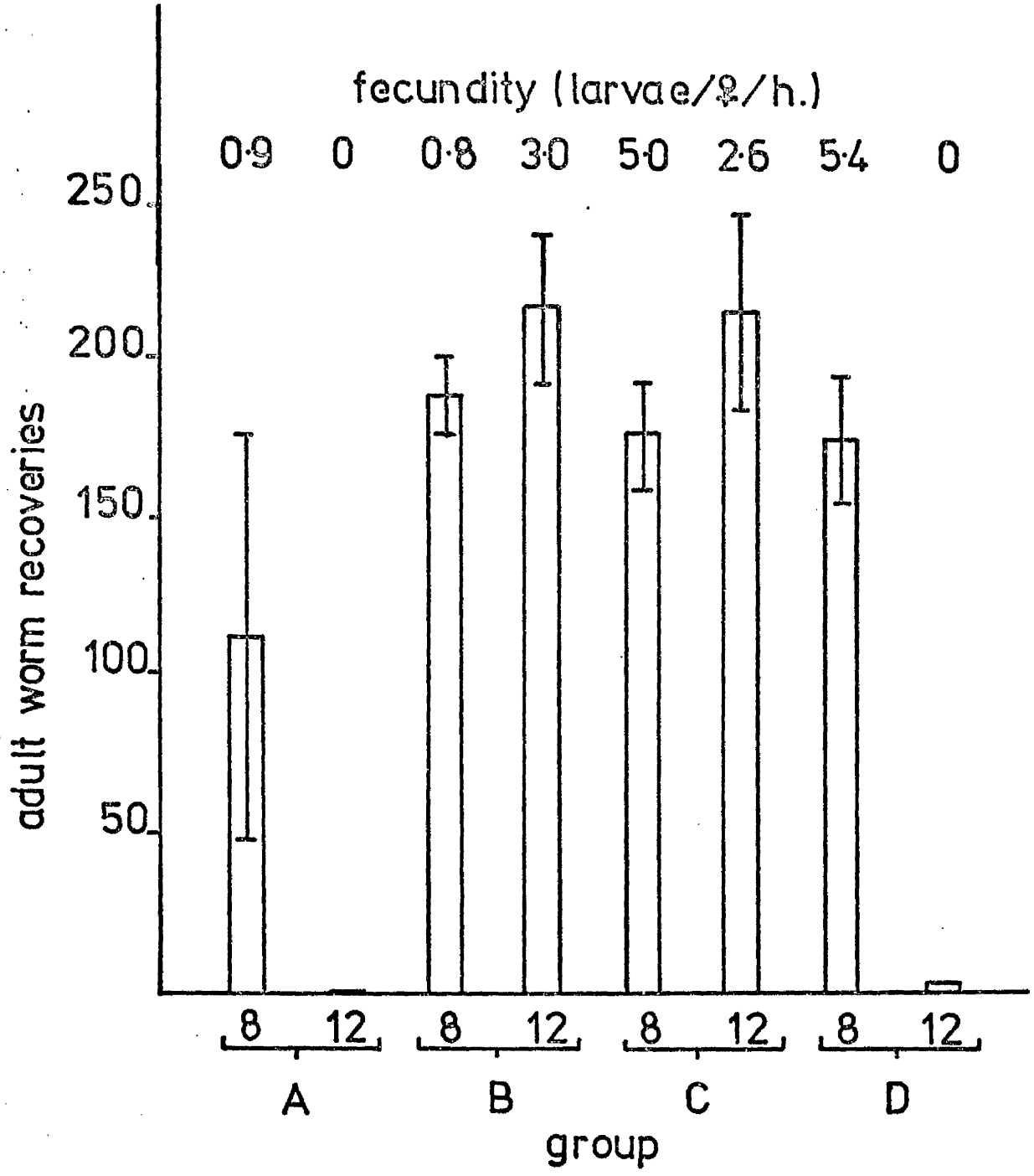
FIGURE 7

Mean recovery (MWR $\pm$ SD) T. spiralis on days 8 and 12 of infection after transfer of MLNC

DONOR		RECIPIENT	GROUP
Tsp	2×10^7 MLNC +	Tsp	A
	2×10^7 MLNC +	Tsp + Nd	B
		Tsp + Nd	C
		Tsp only	D


fecundity (larvae/♀/h.)
0·9
0
0·8
3·0
5·0
2·6
5·4
0
250
200
150
100
50
adult worm recoveries
8
12
8
12
8
12
8
12
A
B
C
D
group

test whether or not the failure to transfer immunity from doubly infected donors was due to a delay in the development of the cells capable of causing worm expulsion two experiments (Experiments 7 and 8) were set up to examine the effect of transferring cells from single and double infection donors, on day 8, as previously described, and also on day 20 when worm expulsion in the double infection groups is completed. The experimental design was similar in both experiments and is outlined below:

DONORS		RECIPIENTS	GROUP
Nd x Tsp	3×10^7 MLNC	+ Tsp	A
Tsp	3×10^7 MLNC	+ Tsp	B
		Tsp only	C
Tsp (Nd, day 6)	3×10^7 MLNC	+ Tsp (day 8 cell transfer only)	D

MLNC donors were infected with 300 T. spiralis and/or 300 N. dubius on day 0 (donors for groups A and B) and with 300 T. spiralis on day 0 followed by 300 N. dubius on day 6 (donors for group D). This group was included in an attempt to find out if even a late exposure of MLNC donors to N. dubius was enough to prevent cells transferring immunity. All groups of mice were infected with 320 T. spiralis immediately after cell transfer and killed 8 days later. The results for these experiments are shown in Figure 8 (day 8 cell transfer, Experiments 7 and 8) and 9 (day 20 cell transfer, Experiments 7 and 8).

Unlike the previous experiments the day 8 MLNC from doubly infected donors (N. dubius plus T. spiralis day 0, Figure 8, Group A, Experiments 7 and 8) were successful in transferring immunity to donors and the reduction in worm burden was as good as that obtained

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FIGURE 8

Mean recovery (MWR $\pm$ SD) *T. spiralis* on day 8 after transfer of MLNC

DONORS		RECIPIENTS	GROUP
Nd x Tsp	3×10^7 MLNC	+ Tsp	A
Tsp	3×10^7 MLNC	+ Tsp	B
		Tsp only	C
Tsp Nd	3×10^7 MLNC	+ Tsp	D

1. results from transfer of day 8 MLNC Experiment 7
2. results from transfer of day 8 MLNC Experiment 8

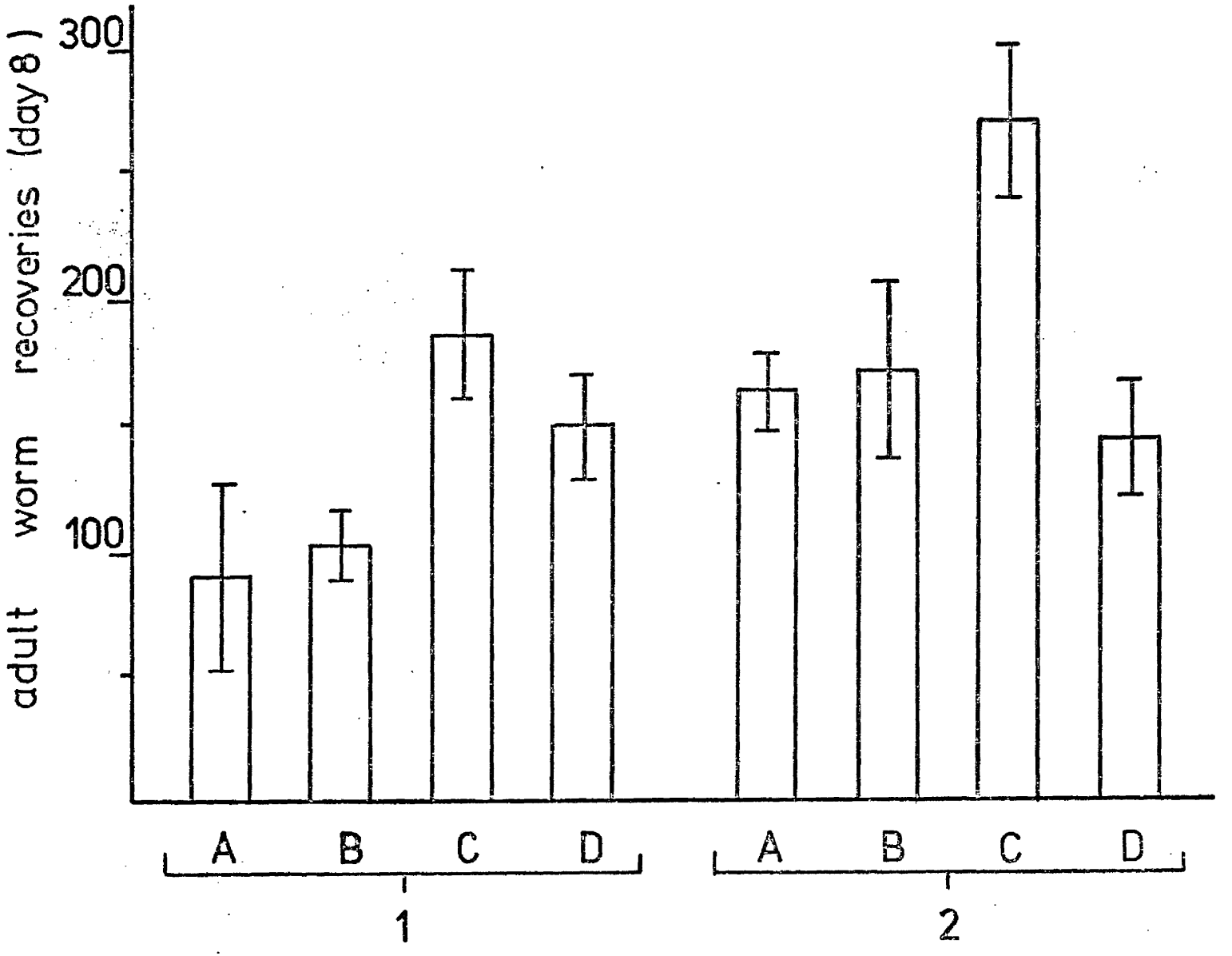
adult worm recoveries (day 8)
300
200
100
A
B
C
D
A
B
C
D
1
2

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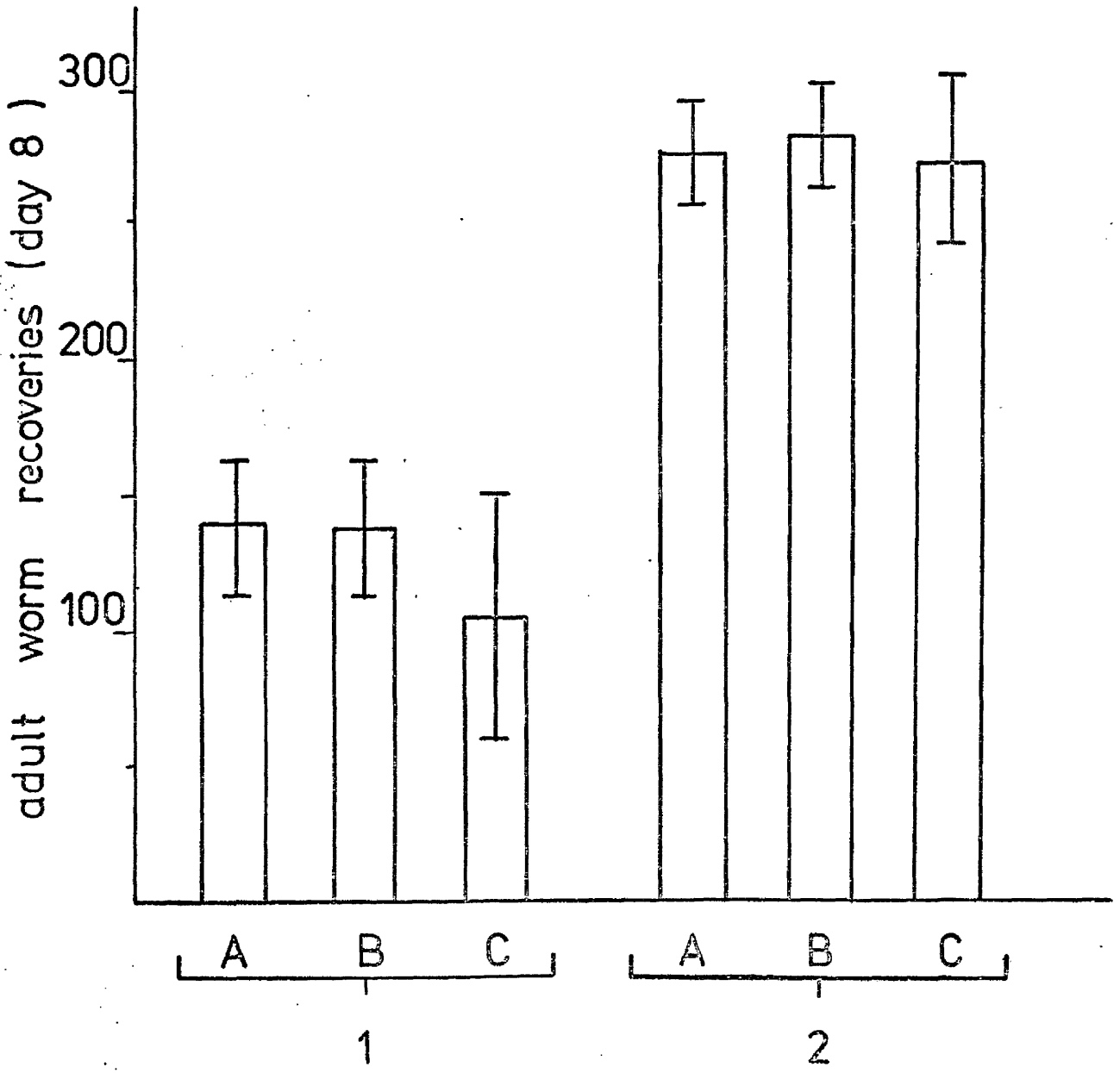
FIGURE 9

Mean recovery (MWR $\pm$ SD) T. spiralis on day 8 after transfer of MLNC

DONORS		RECIPIENTS	GROUP
Nd x Tsp	3×10^7 MLNC +	Tsp	A
Tsp	3×10^7 MLNC +	Tsp	B
		Tsp only	C

1. results from transfer of day 20 MLNC Experiment 7
2. results from transfer of day 20 MLNC Experiment 8


adult worm recoveries (day 8)
300
200
100
A
B
C
A
B
C
1
2

in the MLNC transfer from donors infected with T. spiralis only (Group B). This was consistent in both experiments (see Figure 8). The day 20 results were less consistent and provided anomalous results. The MLNC transfers from single and double infection donors were ineffective in experiments 7 and 8 (see Figure 9). Worm burdens on day 8 post transfer in experiment 8 were higher than would be expected from the infection levels used.

The cell transfers from the mice infected with T. spiralis day 0, followed by N. dubius on day +6, were partially effective in experiment 7 with worm burdens down by 20% compared to controls (Group C), and even more successful in experiment 8 with a 47% reduction in control mean worm burden.

IS THE SUPPRESSION CAUSED BY N. DUBIUS DUE TO THE ACTION OF A SUPPRESSOR CELL?

In the experiments conducted so far the failure of immune MLNC to transfer immunity when they had been taken from 'immune', infected donors or transferred to mice infected with N. dubius has been clearly demonstrated. One way in which suppression could be brought about is for the parasite to alter conditions within the host which favour the production or activity of suppressor cells, these cells would then interfere with the production or activity of other 'immune' cells. To test this the following experiment (Experiment 9) was done.

Donor mice were infected with 300 N. dubius and seven days later single cell suspensions were prepared from the spleen and mesenteric lymph nodes. These cells were injected into recipients (3×10^7 / recipient) as shown in the protocol below:

DONOR		RECIPIENT	GROUP
Nd	3×10^7 MLNC	+ Tsp	A
	3×10^7 SC	+ Tsp	B
		Tsp only	C

Groups of recipients were killed 8 days and 12 days post transfer/infection. Mean worm recoveries are shown in Figure 10. This experiment shared controls with those also shown in Figure 6.

The results indicate that day 8 MLNC/SC from N. dubius infected mice had no effect on the expulsion pattern of T. spiralis in NIH mice (Groups A and B). The worm recoveries resembled closely those of the control (T. spiralis only) group C on both days 8 and 12. Worm lengths (not shown) were also unaffected by the transfers. Worm fecundities were surprisingly low in the group given MLNC (Group A) being only half of the control value on day 8. That this is the result of the action of MLNC, directly or indirectly, on the worm would seem to be unlikely. There is no evidence to suggest that there is any cross-immunity between N. dubius and T. spiralis.

In the previous experiment MLNC had been transferred from N. dubius infected mice to naive mice which were then infected with T. spiralis. In further experiments to assess whether or not a suppressor cell was involved in the effect of N. dubius on T. spiralis expulsion it was decided that the transfer of MLNC to naive recipients might not have been sensitive enough to detect the suppressor activity (if it existed) and so two experiments (Experiments 10 and 11) using mixed cell populations were performed. In both these experiments MLNC donors were mice which had been infected 7 days previously with either N. dubius or T. spiralis. Recipients received one of these single cell populations or a population obtained by

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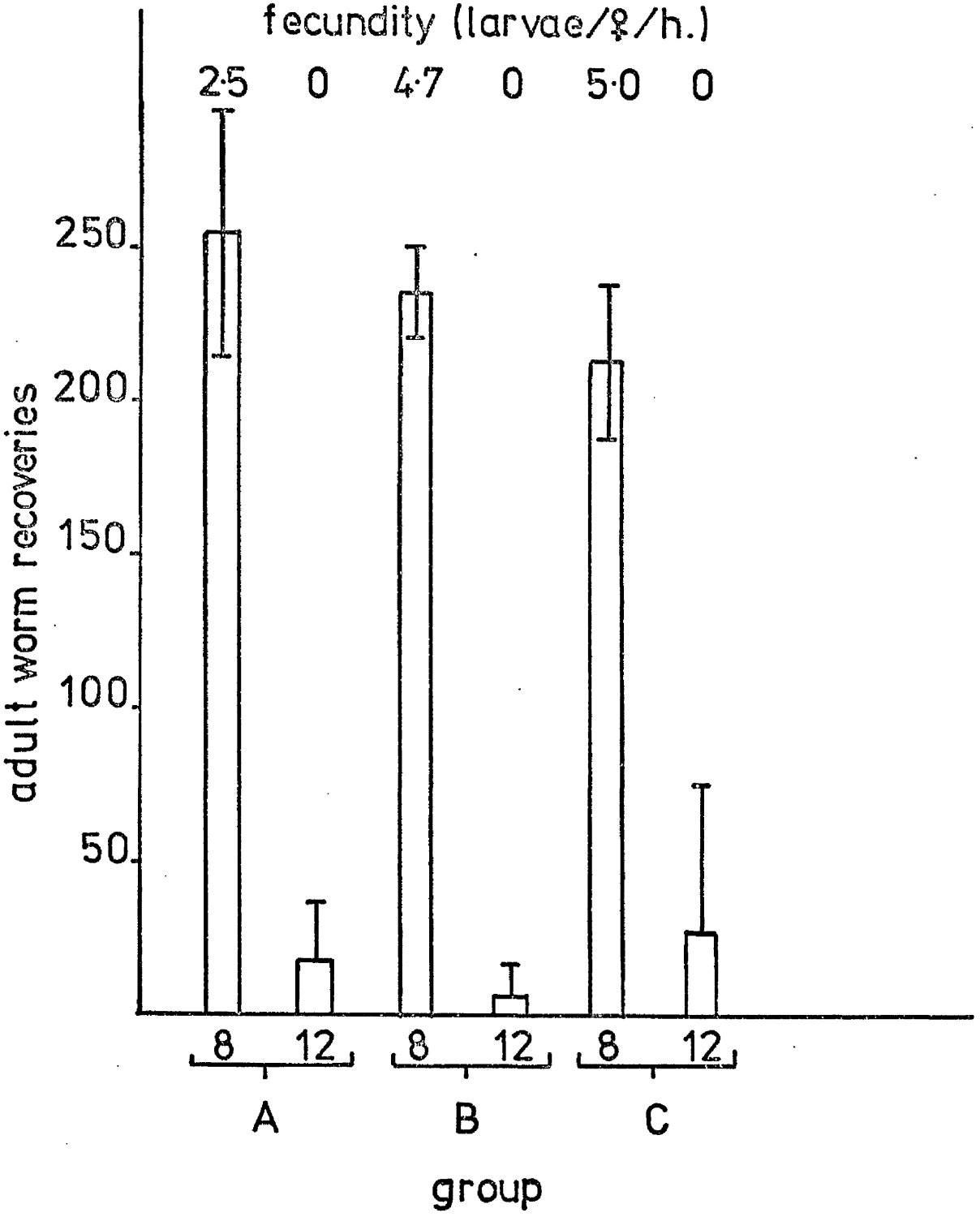
FIGURE 10

Effect of day 7 *N. dubius* MLNC/SC on expulsion of *T. spiralis*

Mean recovery (MWR $\pm$ SD) *T. spiralis* on days 8 and 12 after transfer of MLNC/SC

DONOR		RECIPIENT	GROUP
Nd	3×10^7 MLNC	+ Tsp	A
	3×10^7 SC	+ Tsp	B
		Tsp only	C

Fecundities larvae/female/hour shown above groups.


fecundity (larvae/♀/h.)
2·5
0
4·7
0
5·0
0
250
200
150
100
50
adult worm recoveries
8
12
8
12
8
12
A
B
C
group

mixing the two cell types.

The experimental design for the first experiment (Experiment 10) is set out below:

DONOR		RECIPIENT	GROUP
Nd	1.8×10^7 MLNC	+ Tsp	A
	1.8×10^7 MLNC 2.0×10^7 MLNC	+ Tsp	B
Tsp	2.0×10^7 MLNC	+ Tsp	C
		Tsp only	D

Donors were infected with 300 N. dubius or 276 T. spiralis. All recipient groups plus controls were killed on day 8 post transfer/ infection with 300 T. spiralis. The results are shown in Figure 11. The mean adult worm recovery in group A was similar to that of group D, the control, as was that of group B, the mice which had received the mixed cell population. The mice which had been given only MLNC from T. spiralis infected donors showed the characteristic decline in worm numbers. These results show that the MLNC from N. dubius infected mice prevented the expression of immune MLNC from T. spiralis infected mice.

The second experiment (Experiment 11) is set out below:

DONORS		RECIPIENTS	GROUP
Nd			
	1×10^7 MLNC 1×10^7 MLNC	Tsp	A
Tsp	1×10^7 MLNC	+ Tsp	B
		Tsp only	C

No mice were given N. dubius MLNC only. Donors were infected with 300 N. dubius or 300 T. spiralis and recipients and controls were infected with 276 T. spiralis and killed 8 days post transfer. The

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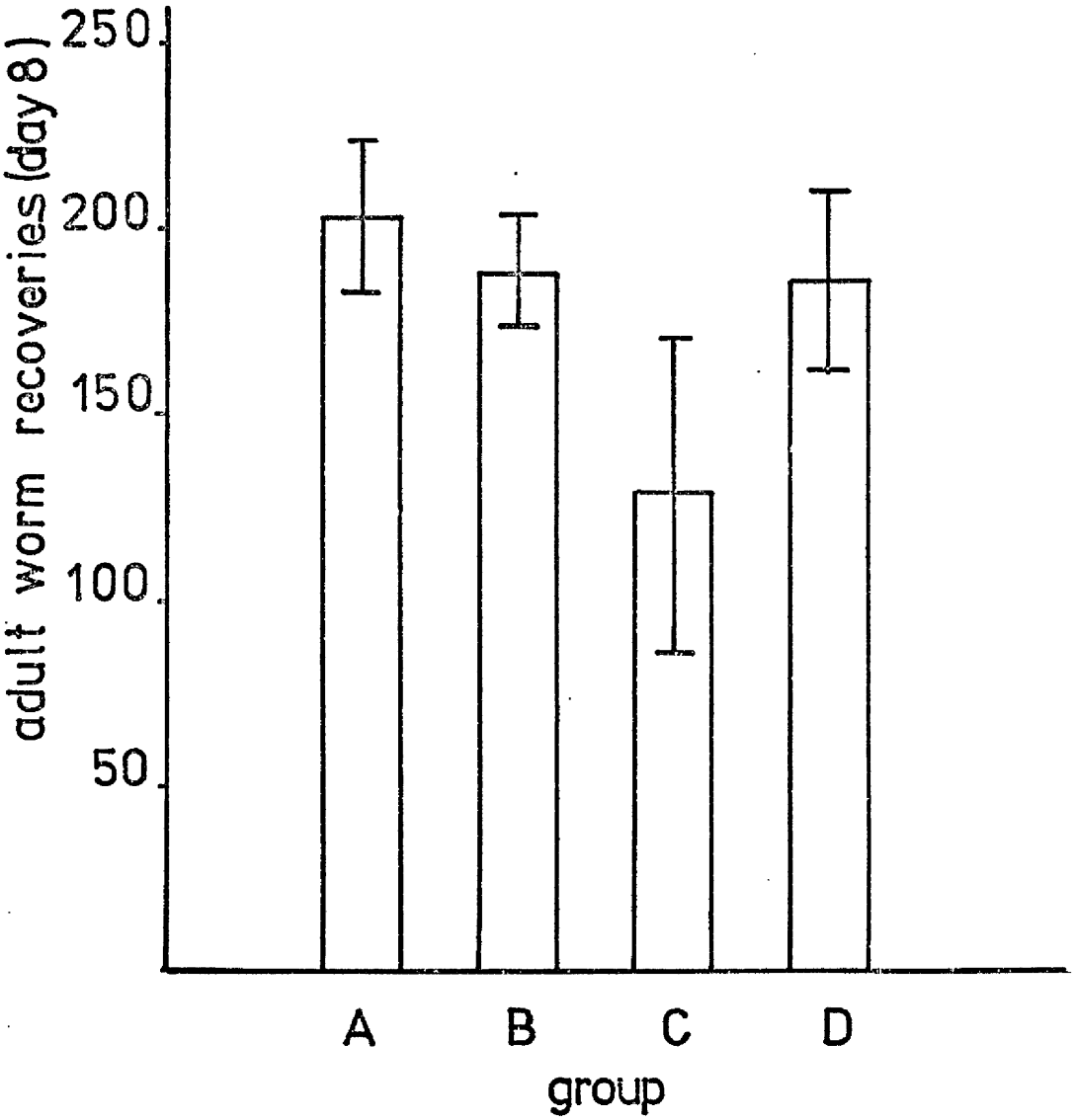
FIGURE 11

Effect of day 7 *N. dubius* and/or *T. spiralis* MLNC on expulsion of *T. spiralis*

Mean recovery (MWR $\pm$ SD) of *T. spiralis* on day 8 of infection after transfer of MLNC

DONOR		RECIPIENT	GROUP
Nd	1.8×10^7 MLNC	+ Tsp	A
	1.8×10^7 MLNC 2.0×10^7 MLNC	+ Tsp	B
Tsp	2.0×10^7 MLNC	+ Tsp	C
		Tsp only	D


adult worm recoveries (day 8)
250
200
150
100
50
A
B
C
D
group

mean worm recoveries are shown in Figure 12. The reduction obtained in group B as a result of the transfer of MLNC from T. spiralis infected mice was not as great as would normally be expected, being only a 23% reduction on the control group (Group C). Once again however the mice given a mixed cell population did not respond as well to the infection although in this case the difference was not statistically significant. From the results obtained here it is difficult to pinpoint the action of an N. dubius induced suppressor cell and to say that this is responsible for the delayed rejection of T. spiralis in doubly infected mice.

The results presented in all these experiments demonstrate clearly that N. dubius can affect the action of MLNC from T. spiralis infected donors. Whilst N. dubius was present MLNC failed to operate within the normal time span. In the penultimate experiment in this series it was decided to expose the donor MLNC to an N. dubius infection for 14 days after they had been transferred to recipients. After this time, and before infection with T. spiralis, the N. dubius infection would be terminated leaving the way open for the MLNC to operate in the normal way. Of course this would only happen if the effect of N. dubius on the transferred MLNC was lost when the N. dubius infection was drug terminated.

The experimental design is set out below:

DONOR		RECIPIENT	GROUP
Tsp	4×10^7 MLNC + 300 Nd	+ Tsp	A
	4×10^7 MLNC + 300 Nd + PYR + PYR	+ Tsp	B
	4×10^7 MLNC	+ Tsp	C
	300 Nd + PYR + PYR	+ Tsp	D
		Tsp only	E

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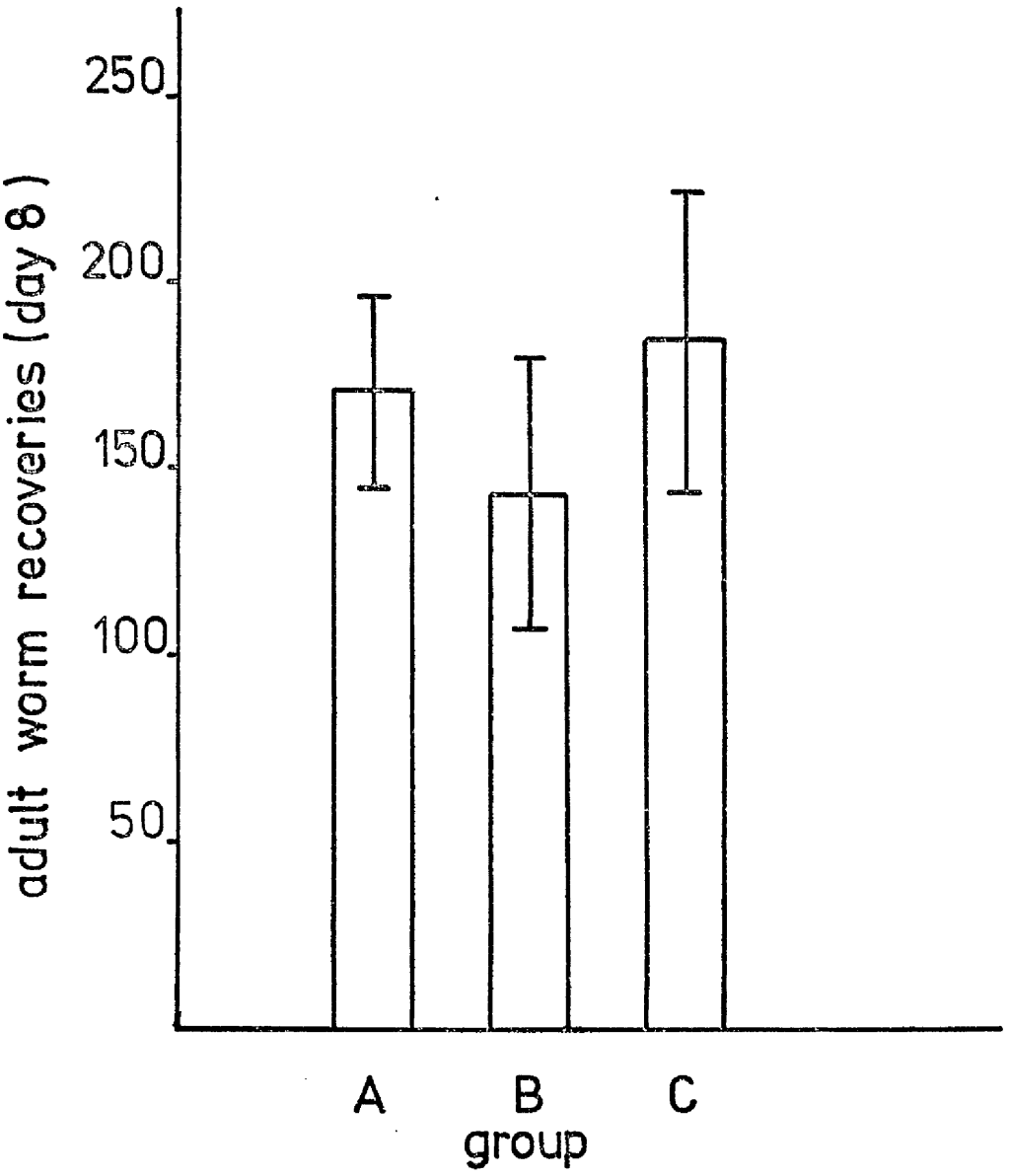
FIGURE 12

Effect of day 7 *N. dubius* and/or *T. spiralis* MLNC on expulsion of *T. spiralis*

Mean recovery (MWR ± SD) of *T. spiralis* on day 8 of infection after transfer of MLNC

DONORS		RECIPIENTS	GROUP
Nd	1×10^7 MLNC 1×10^7 MLNC	+ Tsp	A
Tsp	1×10^7 MLNC	+ Tsp	B
		Tsp only	C


250
200
150
100
50
adult worm recoveries (day 8)
A
B
C
group

Donors, 6-8 week old male NIH mice were infected with 370 T. spiralis 7 days prior to cell transfer. All recipients were infected with 300 T. spiralis, 28 days after cell transfers, and killed 8 days later. N. dubius was given on the same day as the MLNC transfers and in appropriate groups (Groups B and D) terminated by pyrantel treatment on days 14 and 20 of the infection. Results for this experiment (Experiment 12) are shown in Figure 13.

In Group A there was no evidence to suggest that MLNC had been effective, the infection with T. spiralis had been delayed to one month after cell transfer. Group C showed the mean worm recovery from an effective cell transfer and this was more than 60% down on the control values of Group E. In Group D, the removal of the N. dubius infection did not appear to alter the immunosuppressive effect of N. dubius on the T. spiralis infection whereas the removal of the N. dubius infection in the MLNC transfer group, Group B, did have some effect. If we compare Group B with Group D then it appears that the MLNC have had a marked effect once the N. dubius infection had been removed but the mean recovery of T. spiralis in Group B was not any lower than that of control Group E.

DOES N. DUBIUS AFFECT T-CELLS OR B-CELLS?

The role of T and B-cells in the immune elimination of intestinal helminths has already been described (see General Introduction). The response against N. dubius has been shown to be T-dependent, however no evidence exists to demonstrate if the suppression induced by the parasite is due to a selective effect on the activity of T-cells or B-cells although most of the antigens against which the suppressive effect have been measured were T-dependent antigens, i.e. antigens which required the presence/activity of T-cells to induce a response.

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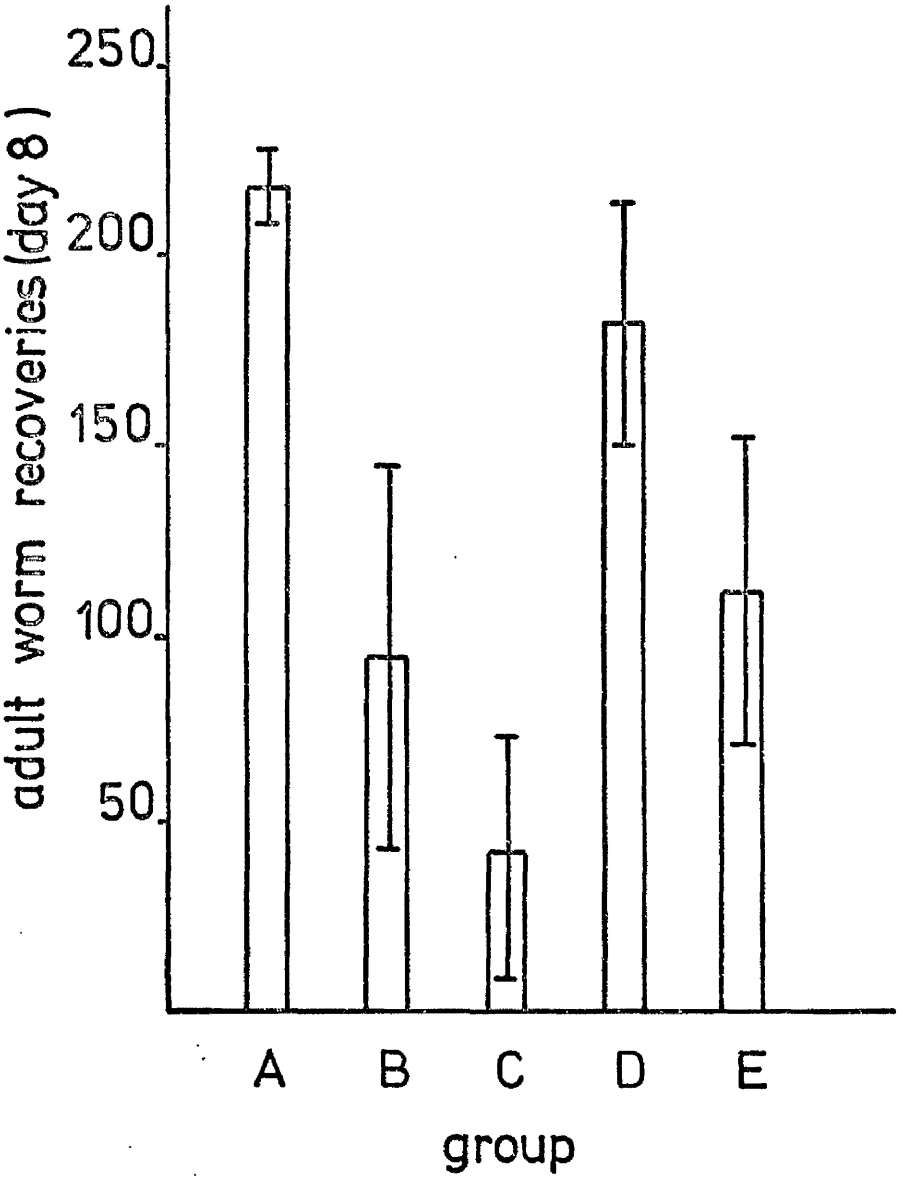
FIGURE 13

Effect of short term exposure of immune MLNC (Tsp) to *N. dubius* infection in recipient mice

Mean recovery (MWR $\pm$ SD) *T. spiralis* on day 8 of infection (36 days after cell transfer)

DONOR	RECIPIENTS	GROUP
Tsp	4×10^7 MLNC + 300 Nd + Tsp	A
	4×10^7 MLNC + 300 Nd + PYR + PYR + Tsp	B
	4×10^7 MLNC + Tsp	C
	300 Nd + PYR + PYR + Tsp	D
	Tsp only	E


250
200
150
100
50
adult worm recoveries (day 8)
A
B
C
D
E
group

Earlier experiments suggested that the fecundity of T. spiralis in MLNC recipient mice was decreased even when worm expulsion was delayed by the presence of an N. dubius infection. This experiment (Experiment 13) was designed to determine which cell population was most affected in its action since it has been suggested that the B-cell population may be responsible for anti-worm effects and the T-cell population for worm expulsion (Wakelin and Wilson 1979).

The cell recipients in this experiment were mice chronically infected with N. dubius. This infection had been present for two months prior to cell transfer/infection. Mice were 14-16 weeks old at the time they received MLNC. The experimental design is set out below:

DONOR			RECIPIENT	GROUP
Tsp			Tsp (Nd)	A
	(T)	MLNC	+ Tsp (Nd)	B
	(B)	MLNC	+ Tsp (Nd)	C
	(T)	MLNC	+ Tsp	D
	(B)	MLNC	+ Tsp	E
		MLNC (unseparated)	+ Tsp	F
			Tsp only	G

All mice (except Group A-G) received a total of 2×10^7 cells. The results are shown in Figure 14. Recipient mice were infected with T. spiralis on the same day as cell transfer. All groups were killed 8 days post transfer/infection. No mice were given unseparated MLNC plus infections with N. dubius and T. spiralis. The mean worm recovery of T. spiralis from Group A, the double infection, was similar to that of control Group G. The T and B-cell transfers to mice infected with T. spiralis (Groups D and E) greatly reduced the mean worm

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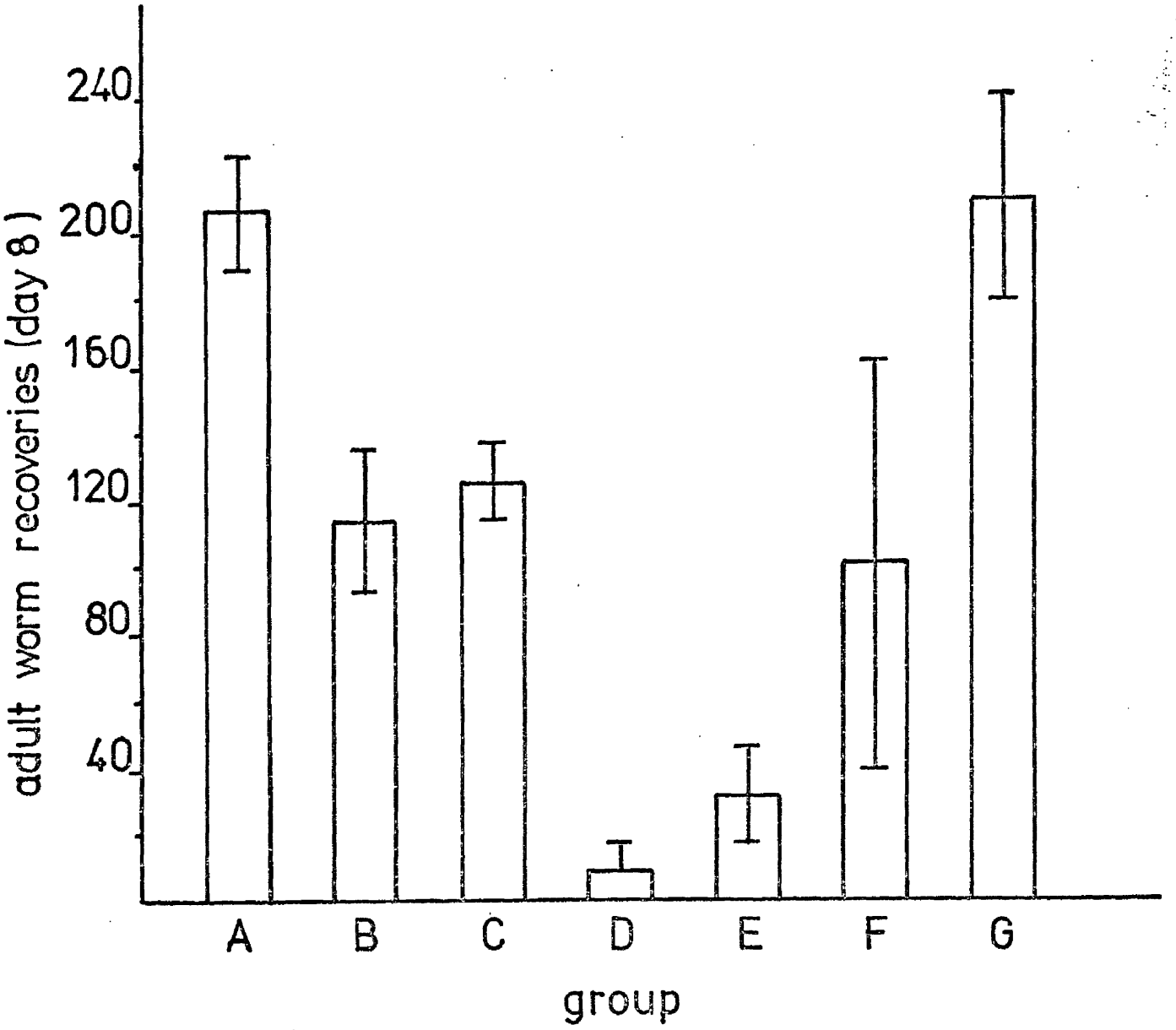
FIGURE 14

Mean recovery (MWR $\pm$ SD) *T. spiralis* on day 8 of infection after transfer of MLNC

DONOR	RECIPIENT	GROUP
Tsp	Tsp (Nd)	A
(T) MLNC +	Tsp (Nd)	B
(B) MLNC +	Tsp (Nd)	C
(T) MLNC +	Tsp	D
(B) MLNC +	Tsp	E
MLNC unseparated +	Tsp	F
	Tsp only	G

Mice in Groups A, B and C harboured an eight week *N. dubius* infection.


240
200
160
120
80
40
adult worm recoveries (day 8)
A
B
C
D
E
F
G
group

recoveries in these groups when compared to the control Group G. This reduction was greater than that obtained with the unseparated MLNC, Group F. However in Groups B and C, the groups given T and B-cells plus infections with T. spiralis and N. dubius the cells were not as effective as the same cells in the single infection groups in reducing the mean worm recovery. Once again there was a marked effect of N. dubius on the effectiveness of MLNC even though the N. dubius infection had been present in the cell recipients for eight weeks. Fecundity values were not measured for Groups D and E. Only Group F showed a significantly decreased fecundity value.

LYMPHOCYTE MIGRATION PATTERNS IN N. DUBIUS/T. SPIRALIS INFECTED MICE

The early experiments (Experiments 1 and 2) presented in section 1(1) showed a marked delay in the expulsion of T. spiralis from mice concurrently infected with N. dubius. As mentioned in the introduction a clear pattern of lymphoid cell homing to the intestine of T. spiralis infected mice has been established. The experiments in this section were designed to find out if a concurrent infection with N. dubius affected lymphoid cell homing properties in T. spiralis infected mice.

The MLNC donors used in the following experiments, were mice which had been infected for 4 days with 300-400 T. spiralis. Day 4 was chosen because at this time a large number of cells can be recovered from the MLNC (Christie 1979). This reduces the number of donors required and provides a cell population which will take up sufficient radio-label to give substantial counts in each organ at recovery. In the first experiment (Experiment 14) labelled cells were transferred to recipients as set out below:

DONOR CELLS	RECIPIENTS	GROUP
Tsp +4 labelled MLNC	Tsp (day 4 of infection)	A
	Nd (day 4 of infection)	B
	Tsp x Nd (day 4 of infection)	C
	Control (no infection)	D

The results in terms of % injected dose of isotope recovered and % of Total recovery per organ are shown in Figures 15 and 16 respectively.

The % recovery of isotope from the small intestine in the day 4 single infection with N. dubius group, (Group B) was similar to that of control Group D, but in mice given the day 4 single infection with

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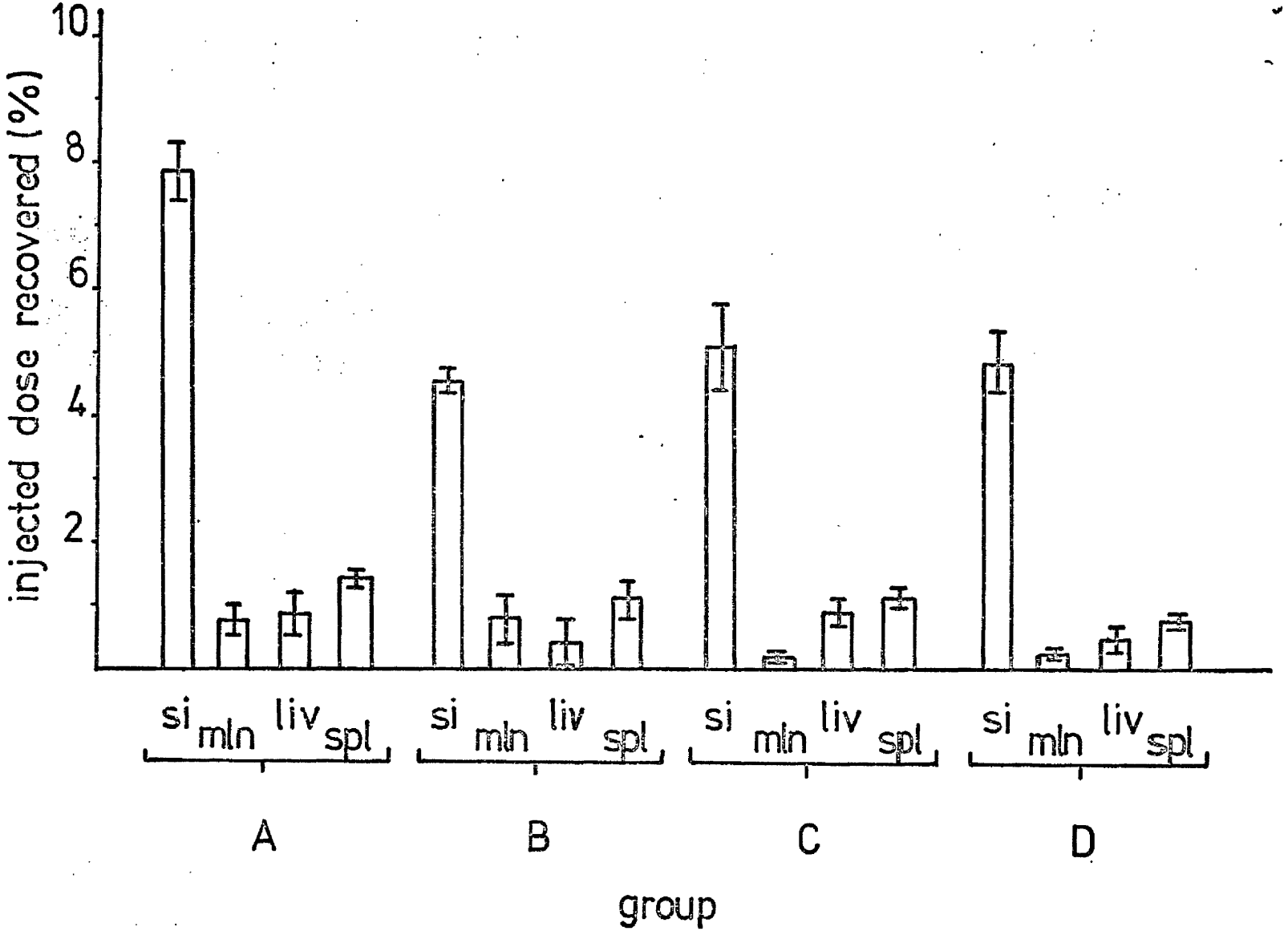
FIGURE 15

Lymphocyte migration patterns in *N. dubius*/*T. spiralis* infected mice

% ID

DONOR CELLS	RECIPIENTS	GROUP
Tsp + 4 labelled MLNC	Tsp (day 4 of infection)	A
	Nd (day 4 of infection)	B
	Tsp x Nd (day 4 of infection)	C
	Control, no infection	D


injected dose recovered (%)
10
8
6
4
2
si
mln
liv
spl
A
B
C
D
group

T. spiralis there was a significant increase over control, with a mean % recovery of 7.82 ± 0.48 (Group A). However the recovery from the small intestine of doubly infected mice, 5.09 ± 0.67, was similar to the control value and significantly lower than T. spiralis alone (Group A). This result demonstrates a marked effect of N. dubius on the lymphoblast homing to the gut of mice concurrently infected with T. spiralis. It is perhaps important to notice that the single infection with N. dubius (Group B) did not significantly decrease homing to the gut, when compared with control values, but did prevent any increase in homing in T. spiralis infected mice. The percentage of label recovered from the MLN was increased in both types of single infection but remained at a low control level in the doubly infected group. All three experimental groups showed increases in spleen counts when compared with the control values. Liver counts were also raised in the T. spiralis/N. dubius group and in the T. spiralis group but not in the N. dubius single infection group. Only T. spiralis infected mice (Group A) showed a significant increase in the total percentage injected dose recovered. The changes in the percentage isotope recovered from each organ, Figure 16, show that the relative distribution of labelled cells in each of the four organs is only slightly affected by the infections. The most significant changes were in the MLN and SI of the day 4 N. dubius infection (Group B). These mice gave lower recoveries than controls from the SI but high recoveries in the MLN. Recoveries were also lower than controls in the SI of T. spiralis (Group A) and N. dubius/ T. spiralis (Group C) infected mice.

The experiment was repeated and additional groups were added to extend the information to day 8 of the T. spiralis and N. dubius infections. This is just prior to the expulsion of T. spiralis in

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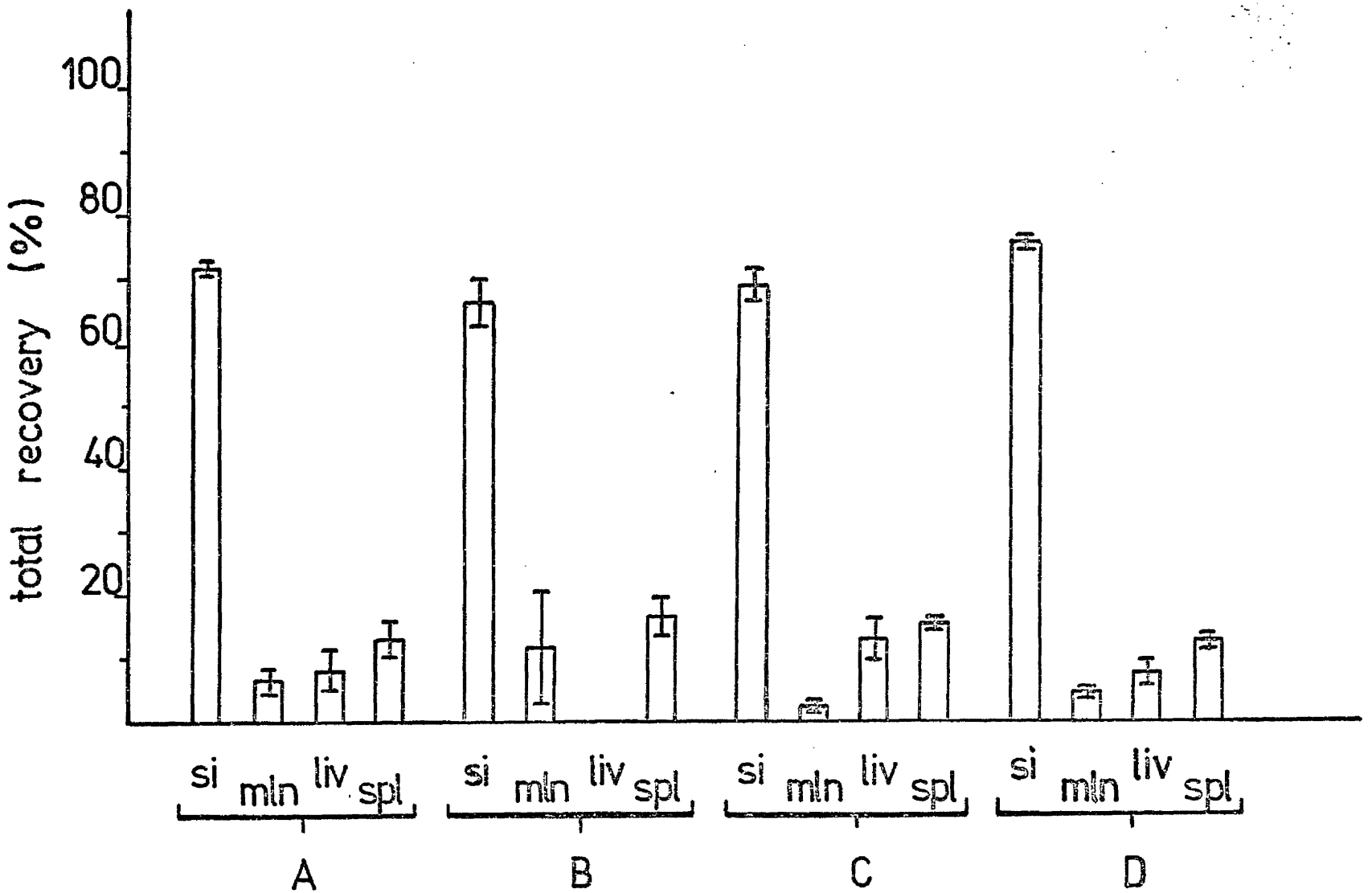
FIGURE 16

Lymphocyte migration patterns in N. dubius/T. spiralis infected mice

% TR

DONOR CELLS	RECIPIENTS	GROUP
Tsp + 4 labelled MLNC	Tsp (day 4 of infection)	A
	Nd (day 4 of infection)	B
	Tsp x Nd (day 4 of infection)	C
	Control, no infection	D


total recovery (%)
100
80
60
40
20
si
mln
liv
spl
A
B
C
D

the normal single infection (see day 8, Figures 1 and 2) and would provide data on whether or not the changes which accompany T. spiralis expulsion, starting on day 8, also occur in the double infection groups in which expulsion is delayed (see Figures 1 and 2). The experimental design (Experiment 15) is set out below:

DONOR CELLS	RECIPIENTS		GROUP
Tsp	Tsp	(day 4 of infection)	A
	Nd x Tsp	(day 4 of infection)	B
	Nd	(day 4 of infection)	C
	Control	(no infection)	D

DONOR CELLS	RECIPIENTS		GROUP
Tsp + 4	Tsp	(day 4 of infection)	A
	Tsp	(day 8 of infection)	B
	Nd	(day 8 of infection)	C
	Nd x Tsp	(day 8 of infection)	D
	Control	(no infection)	E

Because of the problems of processing large numbers of cells for labelling the experiment was carried out on two days but using mice from the same batch. A day 4 T. spiralis infection was included as a positive control (see Figure 18, Group A). The results are shown in Figures 17 and 18 (% injected dose of isotope recovered on days 4 and 8 respectively) and Figures 19 and 20 (% radioactivity recovered per organ on days 4 and 8 respectively).

The day 4 results (see Figures 17 and 19) followed the pattern which had been set in the previous experiment. The percentage injected dose recovered from the small intestine was increased markedly in the T. spiralis day 4 single infection group (Group A, Figure 17).

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FIGURE 17

Lymphocyte migration patterns in *N. dubius*/*T. spiralis* infected mice

% ID

DONOR CELLS	RECIPIENTS	GROUP
Tsp + 4	Tsp (day 4 of infection)	A
	Nd x Tsp (day 4 of infection)	B
	Nd (day 4 of infection)	C
	Control (no infection)	D

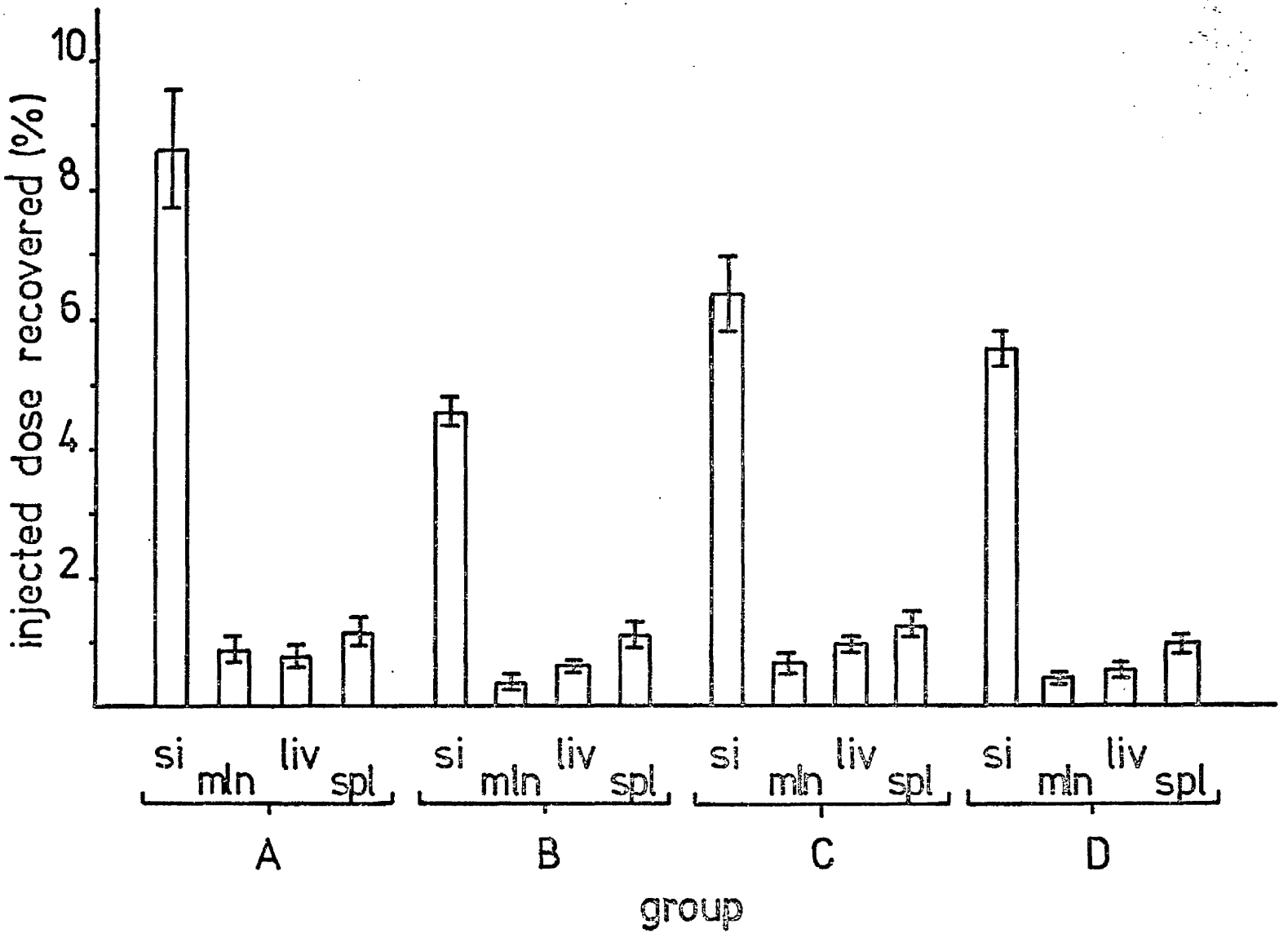
injected dose recovered (%)
10
8
6
4
2
si
mln
liv
spl
A
B
C
D
group

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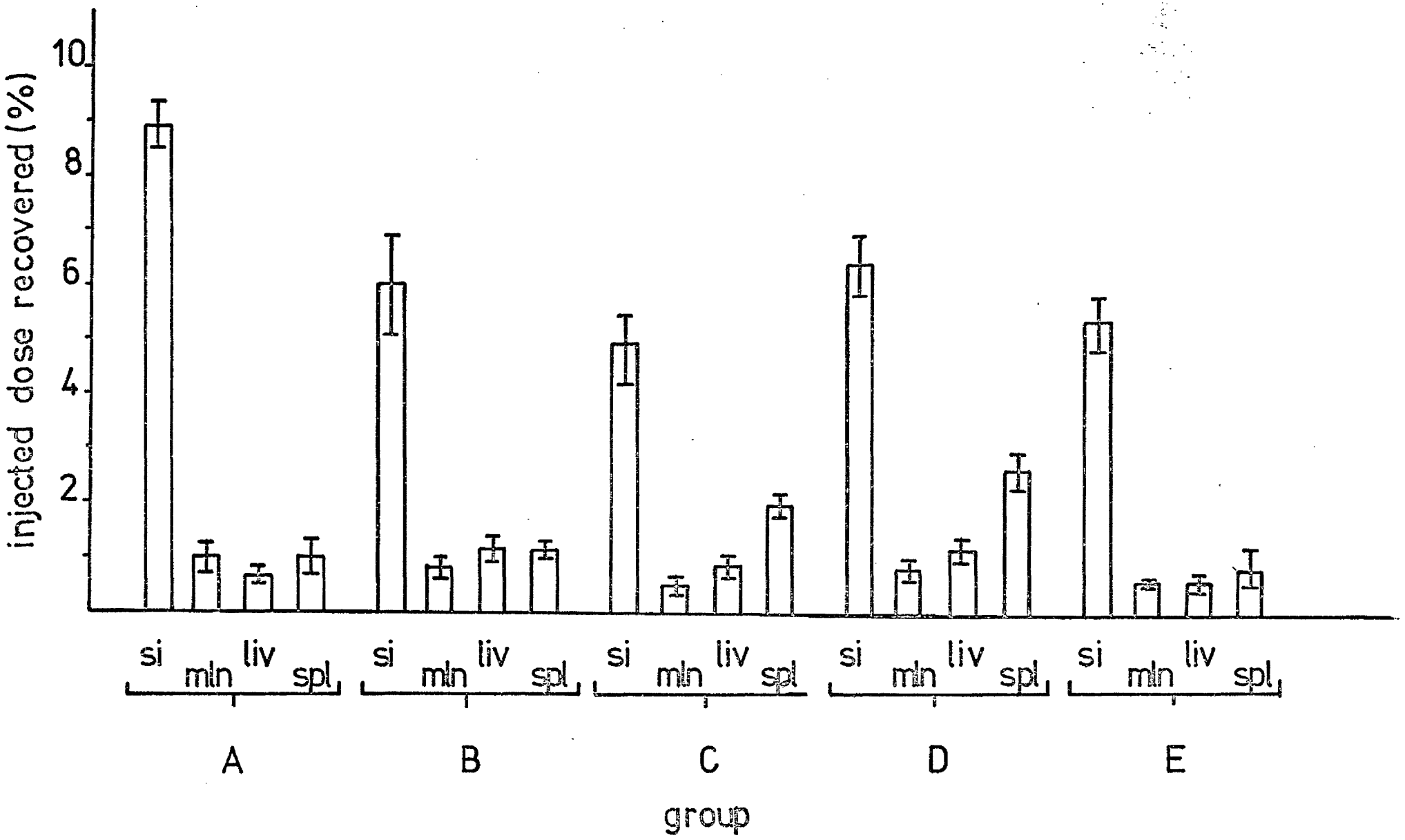
FIGURE 18

Lymphocyte migration patterns in *N. dubius*/*T. spiralis* infected mice

% ID

DONOR CELLS	RECIPIENTS		GROUP
Tsp + 4	Tsp	(day 4 of infection)	A
	Tsp	(day 8 of infection)	B
	Nd	(day 8 of infection)	C
	Nd x Tsp	(day 8 of infection)	D
	Control	(no infection)	E


injected dose recovered (%)
10
8
6
4
2
si
mln
liv
spl
A
B
C
D
E
group

There was also a slight though not significant increase in the double infection group (Group C, Figure 17) and, on this occasion, a significant drop in the label recovery in N. dubius infected mice (Group B, Figure 17). The increase in the recovery from the small intestine in the double infection group was not as great as the increase in the T. spiralis day 4 infection.

The second half of this experiment provided some interesting results. As before, the day 4 single infection small intestine percentage injected dose recovered was significantly greater than control (Figure 18, Group E) but the percentage injected dose recovered from the small intestine of the day 8 single infection (Figure 18, Group B) was only marginally greater than the control value. Although the percentage injected dose recovered from the small intestine of the double infection group on day 8 (Figure 18, Group D) was up by a full one percent (of injected dose) compared with the control value it was still significantly lower than that from the day 4 T. spiralis infection. The recoveries (% injected dose) for the other organs were basically the same in the day 4 groups (Figure 17) as they had been in the previous experiment. There were however, some changes in the day 8 groups, particularly those infected with N. dubius. In both groups (Figure 18, Groups C and D) the counts from the spleen (% injected dose recovered) were at least double those of the control value. This is probably a reflection of the splenomegaly which accompanies N. dubius infection rather than the diversion of labelled cells from the small intestine to the spleen. The changes in percentage total recovery for the spleens in both these groups are shown clearly in Figure 20 (Groups C and D). The results obtained on day 8 might suggest that the failure to detect enhanced migration to the small intestine at this time in double infected mice was due to the diversion

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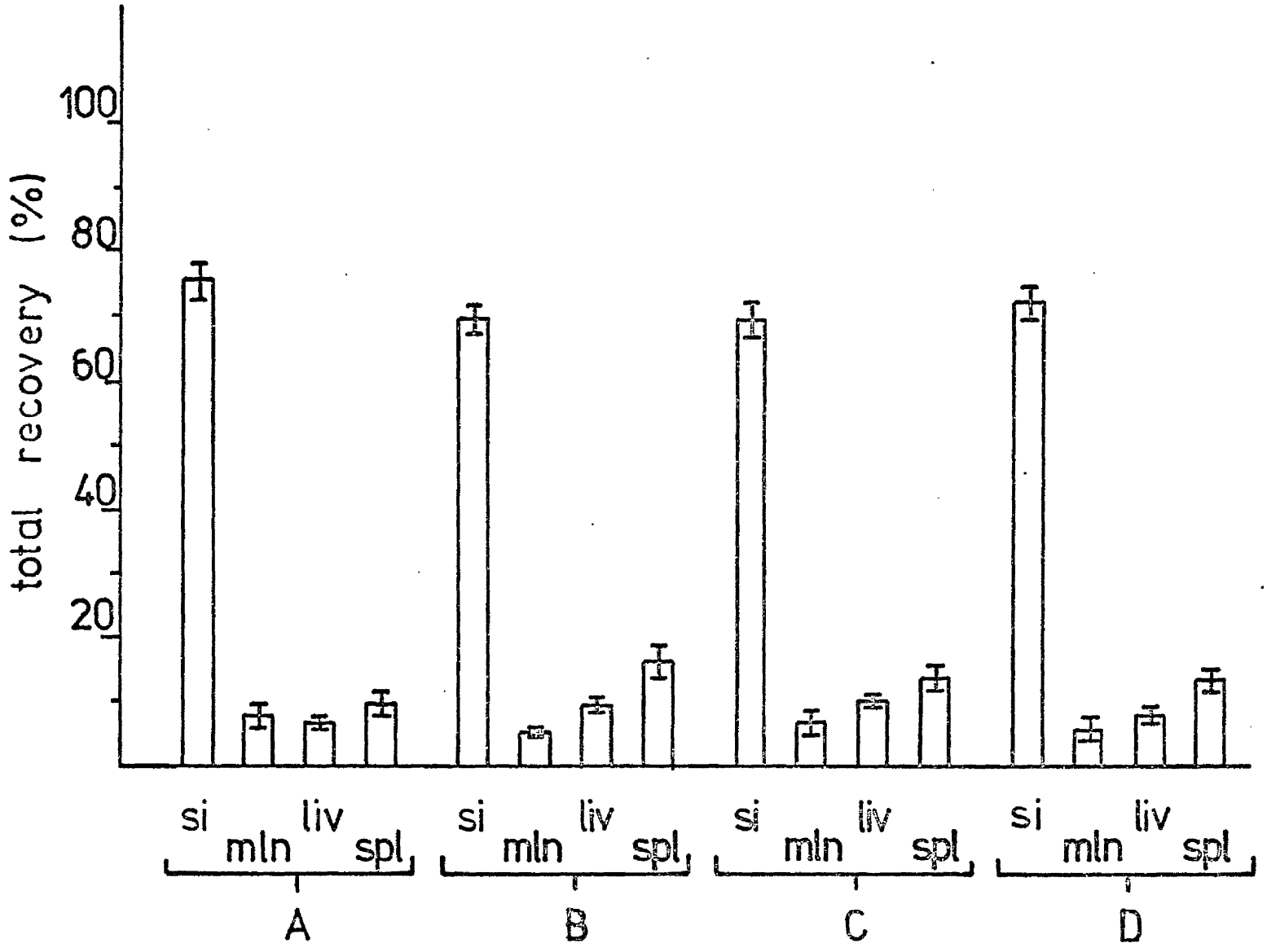
FIGURE 19

Lymphocyte migration patterns in *N. dubius*/*T. spiralis* infected mice

% TR

DONOR CELLS	RECIPIENTS	GROUP
Tsp + 4	Tsp (day 4 of infection)	A
	Nd x Tsp (day 4 of infection)	B
	Nd (day 4 of infection)	C
	Control (no infection)	D


total recovery (%)
100
80
60
40
20
si
mln
liv
spl
A
B
C
D

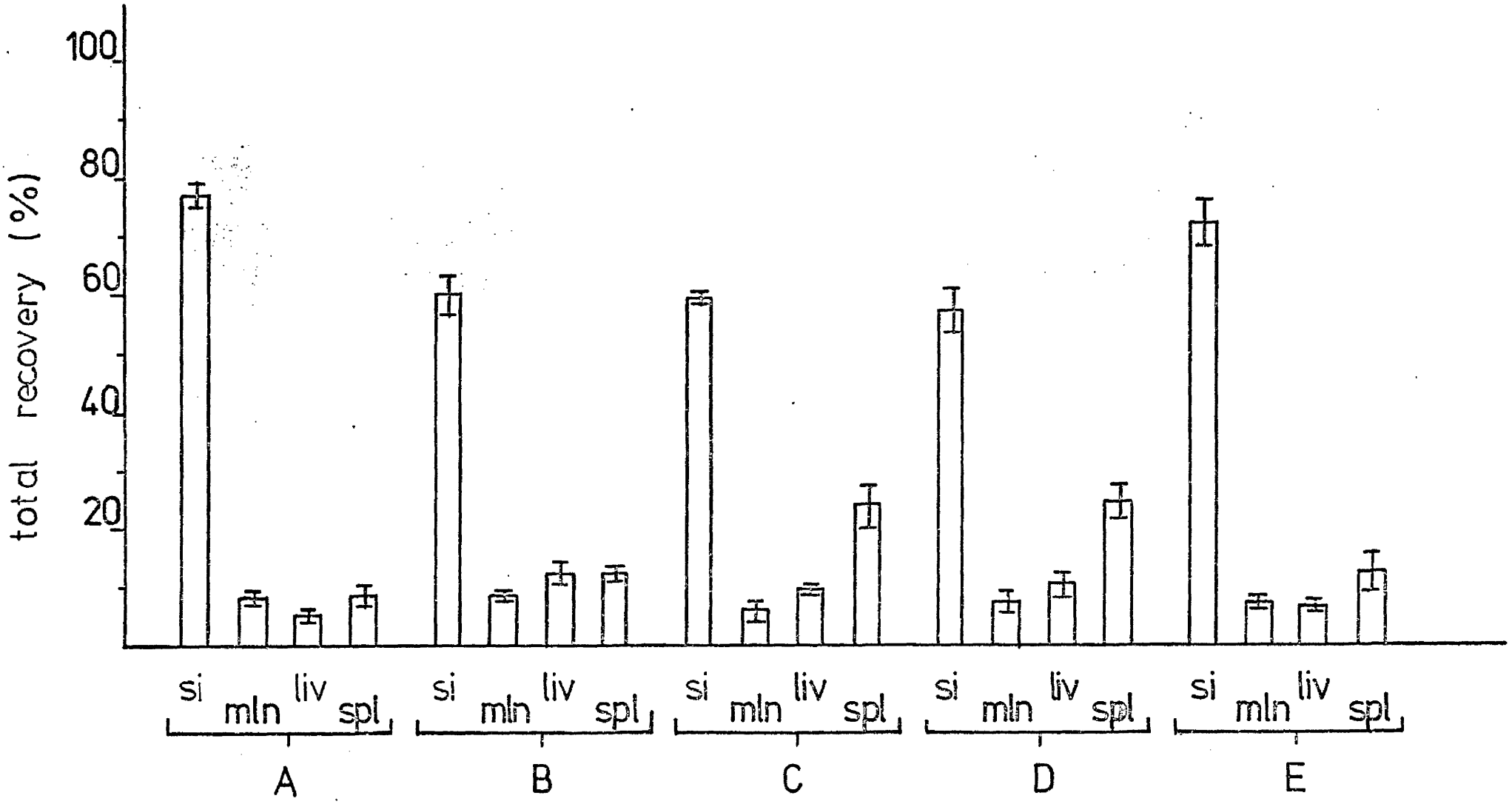
Facing page 71

FIGURE 20

Lymphocyte migration patterns in *N. dubius*/*T. spiralis* infected mice

% TR

DONOR CELLS	RECIPIENTS		GROUP
Tsp + 4	Tsp	(day 4 of infection)	A
	Tsp	(day 8 of infection)	B
	Nd	(day 8 of infection)	C
	Nd x Tsp	(day 8 of infection)	D
	Control	(no infection)	E


total recovery (%)
100
80
60
40
20
si
mln
liv
spl
A
B
C
D
E

of labelled cells from the small intestine to the spleen. However, the suppression of the increased traffic to the intestine seen in doubly infected mice on day 4 was not associated with an increase in the recovery of label from the spleen and thus it would appear unlikely that the failure to detect enhanced migration was due solely to the redistribution of labelled cells from gut to spleen.

The expulsion of T. spiralis from NIH mice is usually complete by day 12 post infection (see Figures 1 and 2) but not if the mice are concurrently infected with N. dubius. The presence of an N. dubius infection delays rather than prevents expulsion. The previous experiments have shown that the increased intestinal recovery (% injected dose) normally associated with day 4 of a T. spiralis infection does not occur in the presence of N. dubius. The next experiment (Experiment 16) examined the lymphoblast migration patterns in mice on day 12 of single and concurrent infections of N. dubius and T. spiralis to determine if enhanced migration, like worm expulsion, was merely delayed in the presence of N. dubius. The experimental design is set out below (Experiment 16):

DONOR CELLS	RECIPIENTS		GROUP
Tsp + 4	Tsp	(day 4 of infection)	A
	Tsp	(day 12 of infection)	B
	Nd	(day 12 of infection)	C
	Tsp x Nd	(day 12 of infection)	D
	Control	(no infection)	E

Once again a group of recipients with a day 4 T. spiralis infection were included as a positive control. The results are shown in Figures 21 (% injected dose of isotope recovered) and 22 (% of Total recovery per organ). The important results, % injected dose recovered,

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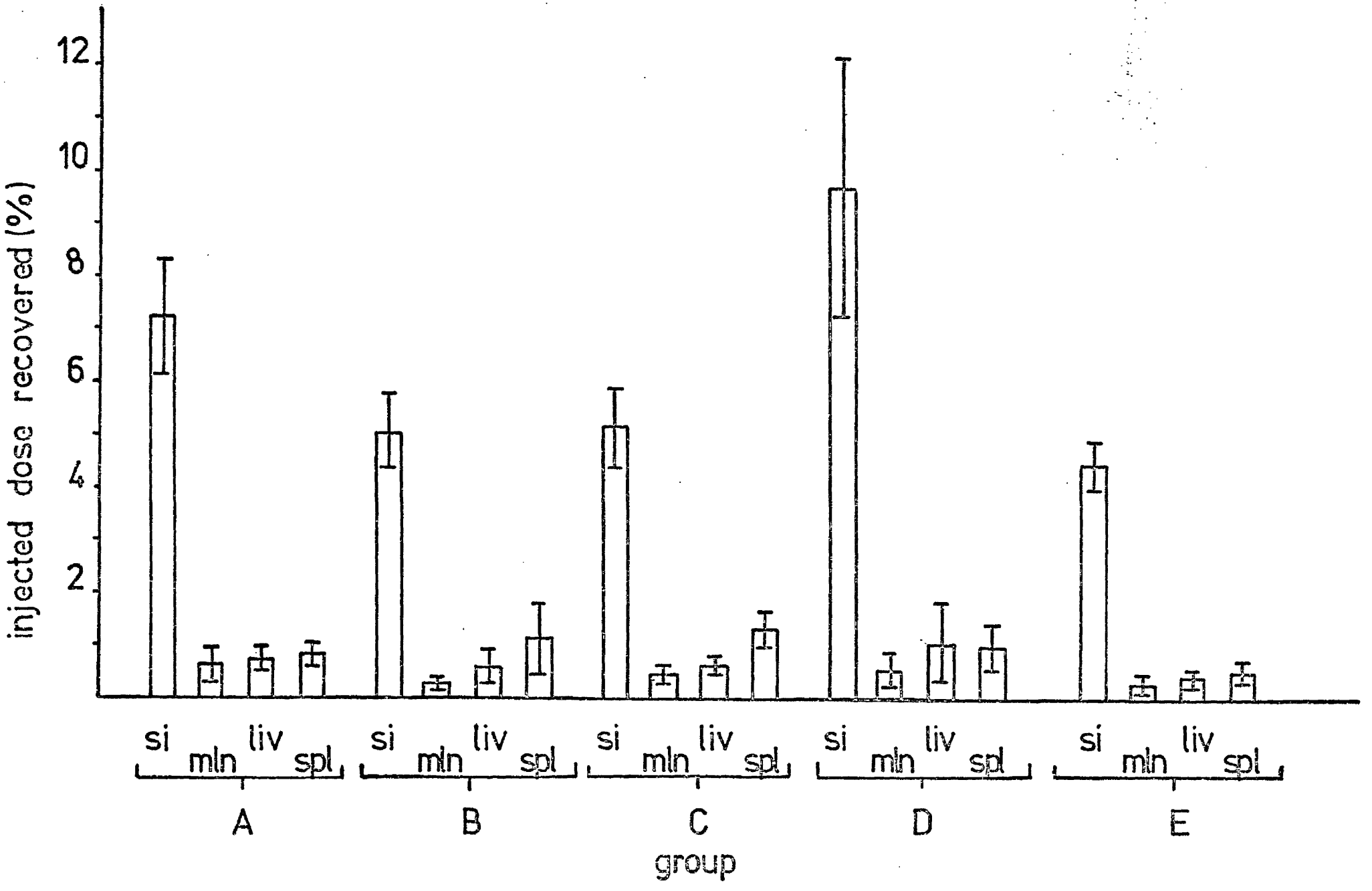
FIGURE 21

Lymphocyte migration patterns in *N. dubius*/*T. spiralis* infected mice

% ID

DONOR CELLS	RECIPIENTS		GROUP
Tsp + 4	Tsp	(day 4 of infection)	A
	Tsp	(day 12 of infection)	B
	Nd	(day 12 of infection)	C
	Tsp x Nd	(day 12 of infection)	D
	Control	(no infection)	E


injected dose recovered (%)
12
10
8
6
4
2
si
mln
liv
spl
A
B
C
D
E
group

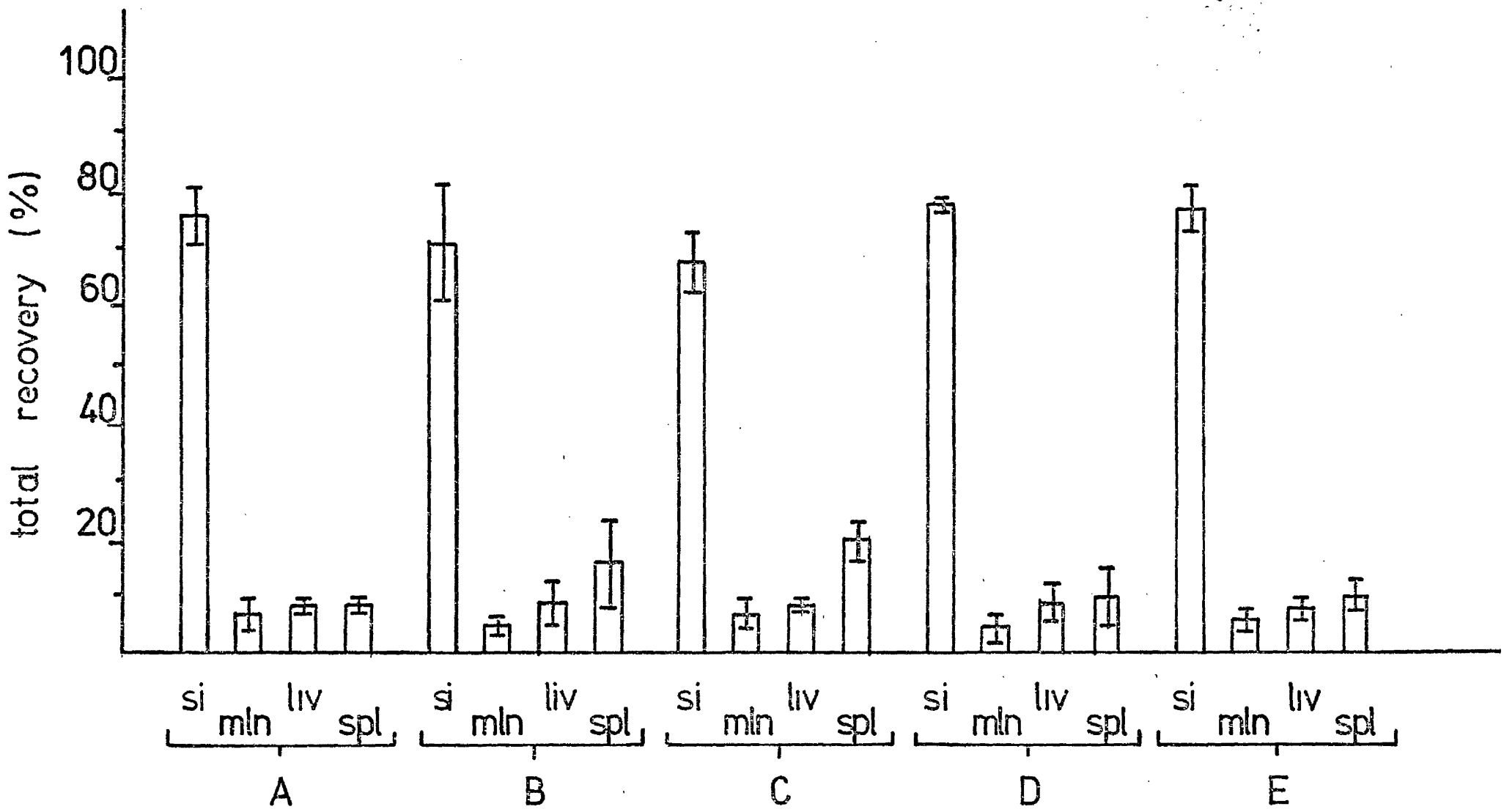
Facing page 74

FIGURE 22

Lymphocyte migration patterns in N. dubius/T. spiralis infected mice

% TR

DONOR CELLS	RECIPIENTS		GROUP
Tsp + 4	Tsp	(day 4 of infection)	A
	Tsp	(day 12 of infection)	B
	Nd	(day 12 of infection)	C
	Tsp x Nd	(day 12 of infection)	D
	Control	(no infection)	E


total recovery (%)
100
80
60
40
20
si
mln
liv
spl
A
B
C
D
E

(Figure 21) are the small intestine values obtained for the day 4 T. spiralis infection (Group A) and the N. dubius x T. spiralis day 12 infection (Group D). The day 4 T. spiralis infection gave a good positive (increased homing) control once again. The double infection group (Group D, Figure 21) showed a massive increase (over control) of the % injected dose of isotope recovered from the small intestine. This increase was greater even than that of the day 4 T. spiralis and N. dubius day 12 single infections (Groups B and C, Figure 21).

Recoveries for the other organs were as before except that the counts from the spleens of all day 12 infected groups were higher than those of controls. Both groups with day 12 N. dubius infections (Groups C and D) were lower than the values obtained for these groups on day 8 of infection (see previous result).

The final experiment in this series (Experiment 17) examined lymphoblast migration patterns on day 16 of single and double infections with N. dubius and T. spiralis. This is the time when the expulsion of T. spiralis in doubly infected mice is about to begin (see Figures 1 and 2). Included in this experiment was a day 4 T. spiralis infection as a positive homing control. Also included was a group of mice which were infected with N. dubius 12 days before T. spiralis infection, the T. spiralis infection being given 4 days prior to the assessment of lymphoblast migration. The gap between infections allowed the N. dubius time to complete development to the adult stage and emerge from the mucosa into the gut lumen before mice were infected with T. spiralis. This group was included to determine if the suppressive effect of N. dubius on enhanced migration to the small intestine of day 4 T. spiralis infected mice (see Group B Figure 17) was reduced when the parasite was in the lumen

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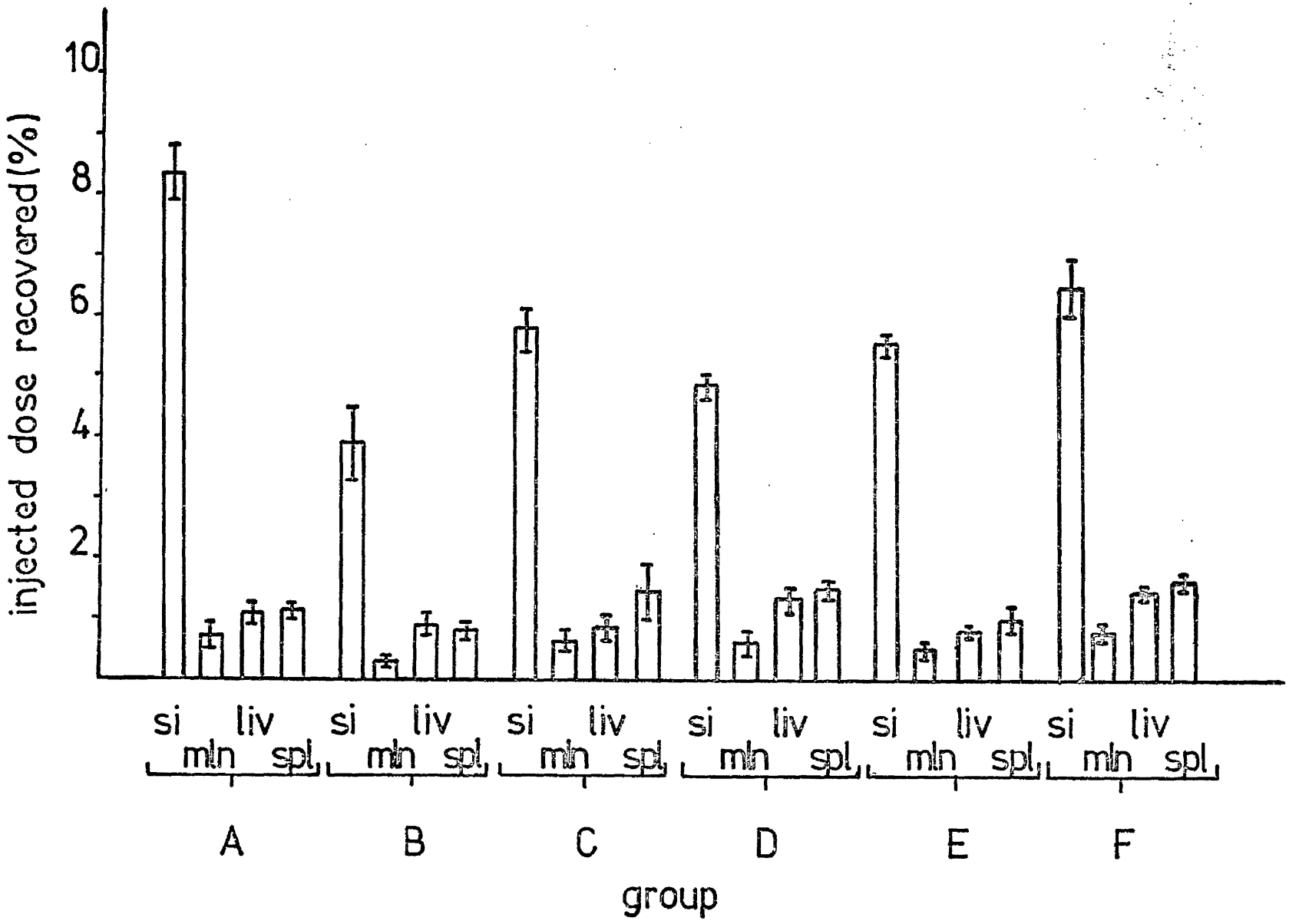
FIGURE 23

Lymphocyte migration patterns in *N. dubius*/*T. spiralis* infected mice

% ID

DONOR CELLS		GROUP
Tsp + 4	Tsp (day 4 of infection)	A
	Tsp (day 16 of infection)	B
	Nd (day 16 of infection)	C
	Nd (day 16) and Tsp (day 16 of infection)	D
	Control (no infection)	E
	Nd (day 12) and Tsp (day 4 of infection)	F


injected dose recovered (%)
10
8
6
4
2
si liv mln spl
si liv mln spl
si liv mln spl
si liv mln spl
si liv mln spl
si liv mln spl
A
B
C
D
E
F
group

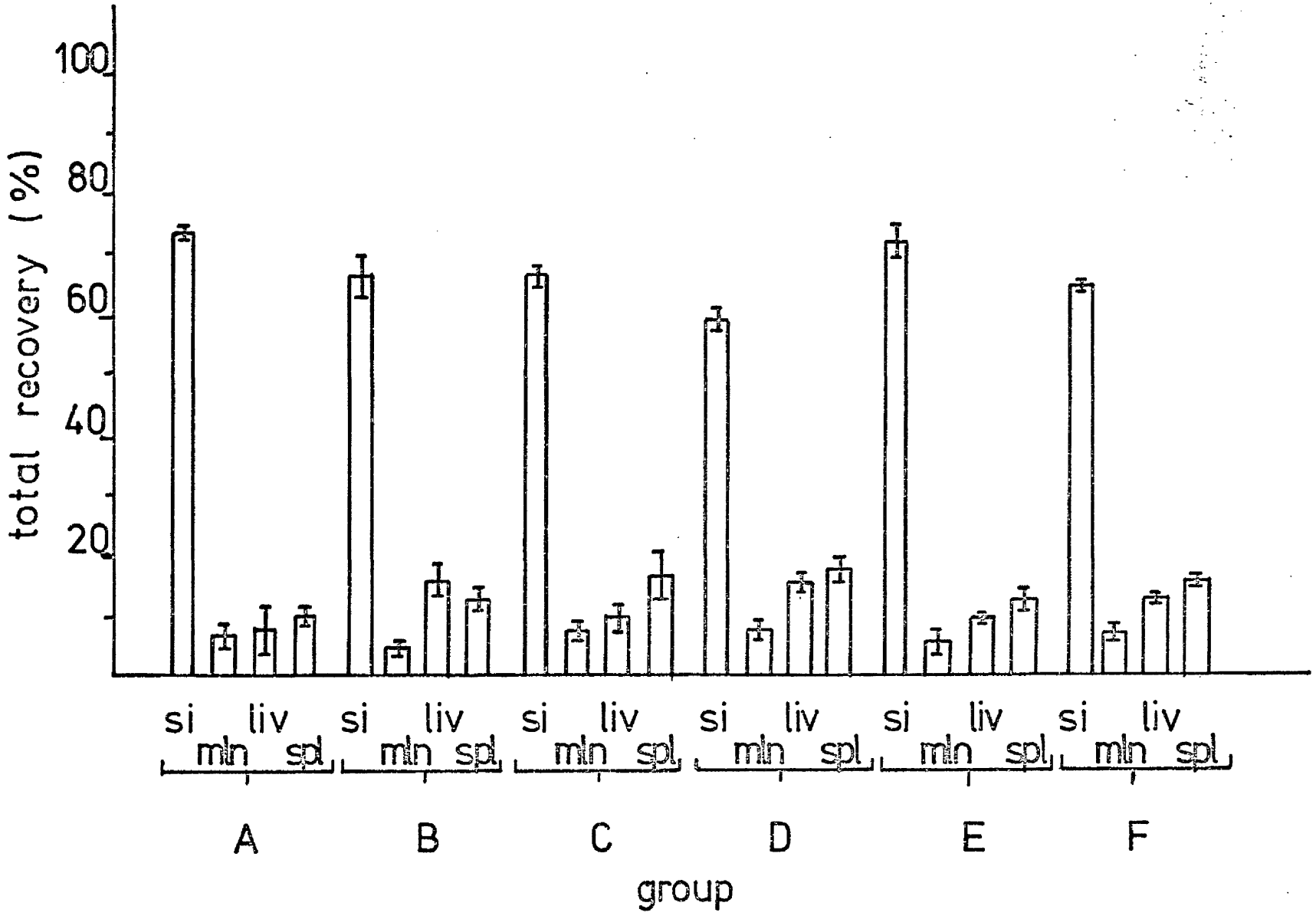
Facing page 77

FIGURE 24

Lymphocyte migration patterns in N. dubius/T. spiralis infected mice

% TR

DONOR CELLS		GROUP
Tsp + 4	Tsp (day 4 of infection)	A
	Tsp (day 16 of infection)	B
	Nd (day 16 of infection)	C
	Nd (day 16) and Tsp (day 16 of infection)	D
	Control (no infection)	E
	Nd (day 12) and Tsp (day 4 of infection)	F


total recovery (%)
100
80
60
40
20
si
liv
mln
spl
A
B
C
D
E
F
group

and secondly to determine if the enhanced migration observed in doubly infected mice (Group D Figure 21) was simply due to the emergence of N. dubius from the intestinal wall. The results are shown in Figures 23 and 24.

Once again the percentage injected dose of isotope recovered from the small intestine on day 4 of the T. spiralis single infection was significantly higher than control (compare Groups A and E, Figure 23). None of the other groups showed such a large increase (small intestine % injected dose of isotope recovered), although the N. dubius day 16/T. spiralis day 4 group (Figure 23, Group F) did show a slight increase over control (Group E). N. dubius day 16/T. spiralis day 16 and T. spiralis day 16 groups (Groups D and B, Figure 23) both gave small intestine recoveries lower than those of controls. The percentage total recovery followed much the same pattern as in previous experiments. The recovery from the small intestine of the N. dubius day 16/T. spiralis day 16 group was much lower than of any other group.

The last figures in this section are the summary tables for each of the organs over the complete period of the study. They give a clearer day to day record of the changes occurring within each organ in the different infection groups. The mean value for the control groups (uninfected mice) ($\pm$ 2 standard deviations) is shown on each table. These results will not be described again but will be referred to in the discussion (Figures 25, 26, 27, 28).

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FIGURE 25

Lymphocyte migration patterns in N. dubius/T. spiralis infected mice

● Tsp

□ Nd

▣ Nd x Tsp

○ Control

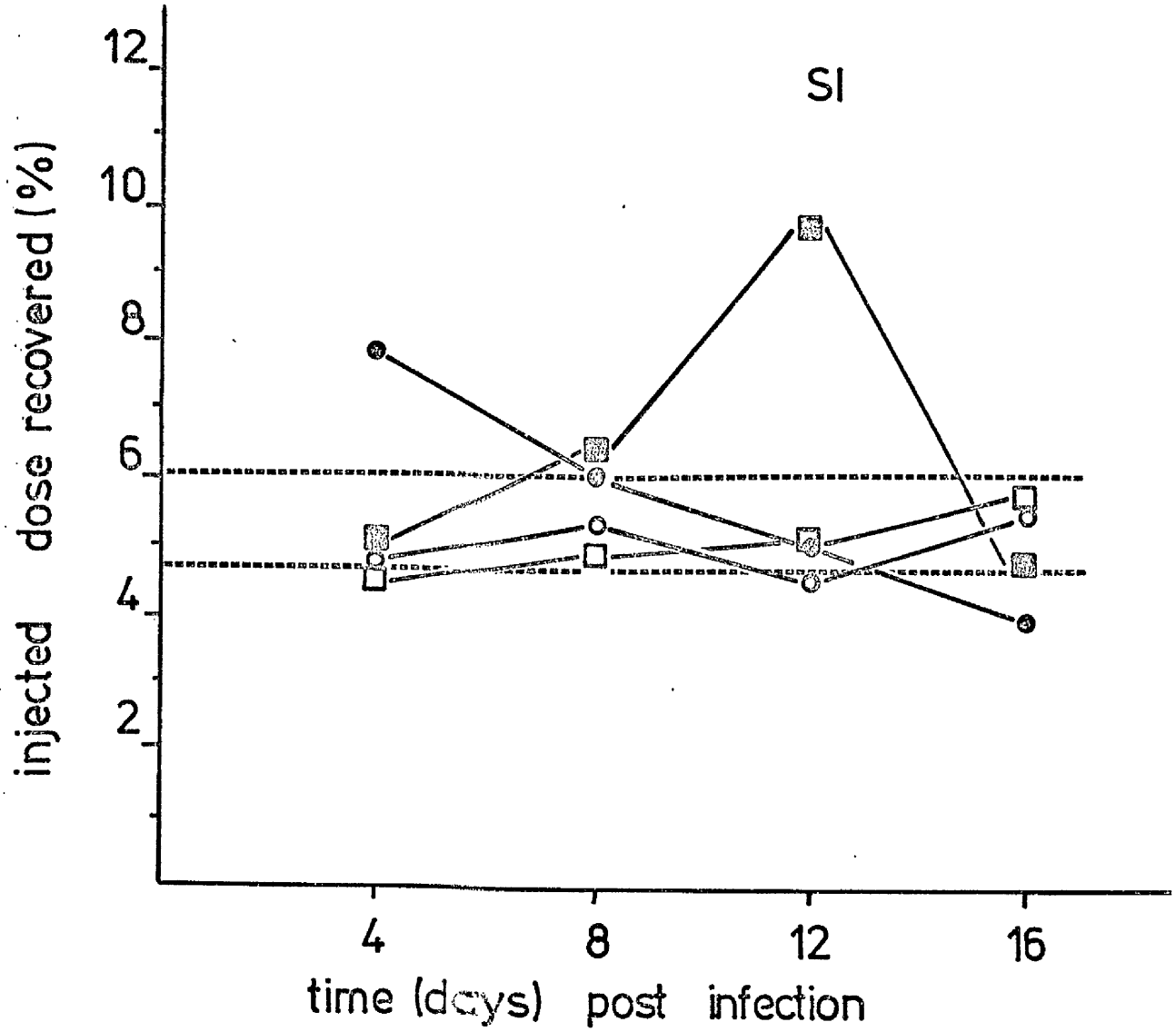
SI
injected dose recovered (%)
12
10
8
6
4
2
4
8
12
16
time (days) post infection

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FIGURE 26

Lymphocyte migration patterns in N. dubius/T. spiralis infected mice

● Tsp

□ Nd

■ Nd x Tsp

○ Control

FIGURE 27

Lymphocyte migration patterns in N. dubius/T. spiralis infected mice

◐ Tsp

□ Nd

◧ Nd x Tsp

○ Control

FIGURE 28

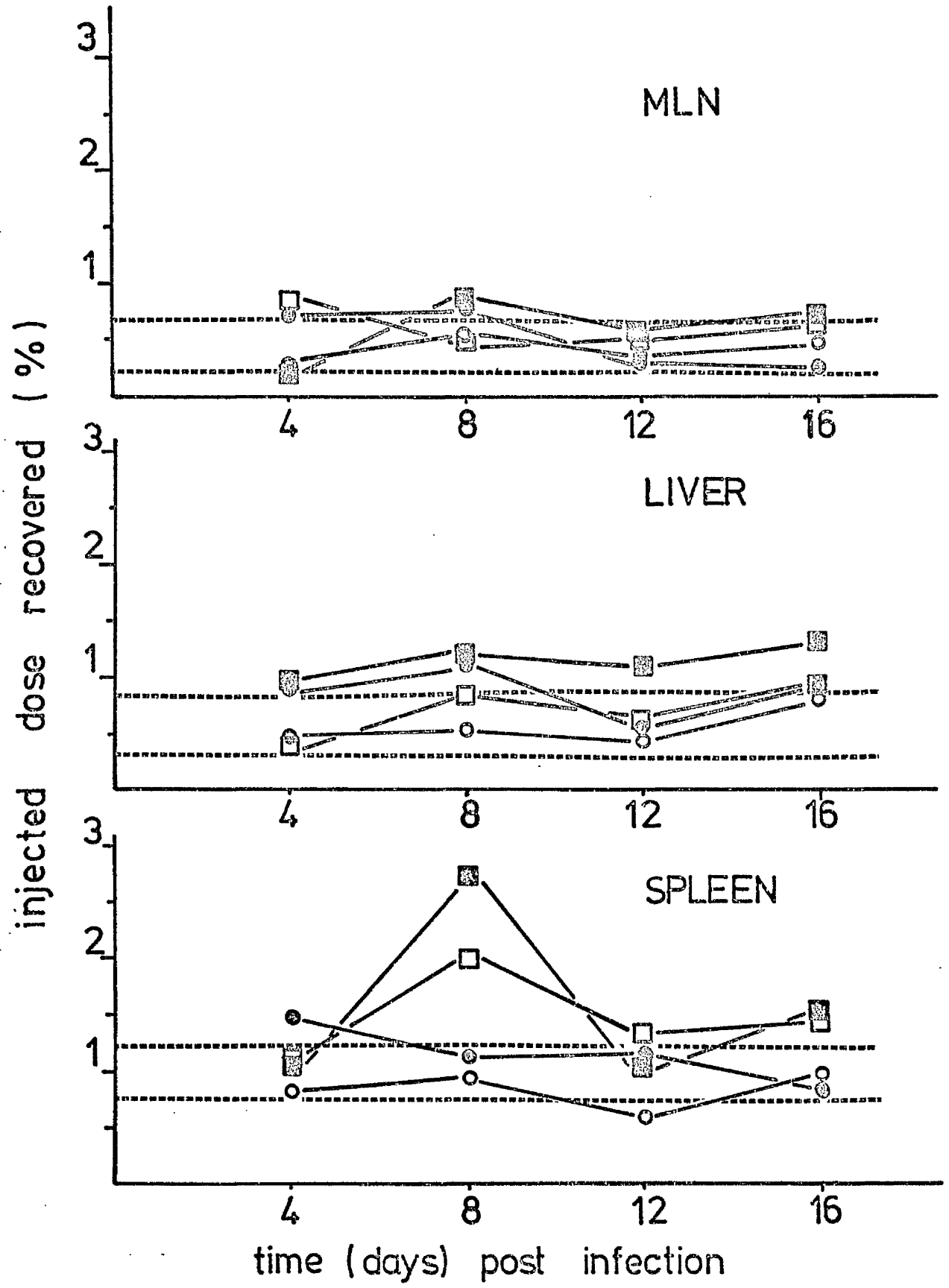
Lymphocyte migration patterns in N. dubius/T. spiralis infected mice

◐ Tsp

□ Nd

◧ Nd x Tsp

○ Control


MLN
LIVER
SPLEEN
3
2
1
4
8
12
16
injected dose recovered (%)
time (days) post infection

Cell responsiveness

during

N. dubius infection

SECTION 1(2)

INTRODUCTION

From the results obtained in the previous section using the *N. dubius*-*T. spiralis*-mouse system there can be little doubt that, during concurrent infection with *N. dubius*, some part(s) of the immune response or the inflammatory response against *T. spiralis* are slow to operate or fail to function altogether. The complexity of the *in vivo* system means that it is not possible to pinpoint the lesion using this approach and accordingly further *in vivo* and *in vitro* studies of cellular responsiveness during *N. dubius* infection were undertaken. Two cell types were examined namely macrophages, because of the important role they play in antigen processing and presentation to T and B lymphocytes and lymphocytes because of their roles in initiation, modulation and expression of the immune response. These two cell types are likely to have the most profound effects on immune responsiveness if their normal functioning is prevented or delayed. Macrophages were examined in an inflammation test, lymphocytes in a mitogen stimulation assay.

Peritoneal macrophages have been seen to adhere to larvae of *N. dubius* both *in vivo* and *in vitro* and it has been postulated that macrophages play a major role in the response to a single infection with *N. dubius* (Chaicumpa and Jenkin 1978). Macrophage activity and functioning can be examined in a variety of ways, the most commonly used methods involving histochemical techniques which are specific for certain lysosomal enzymes such as the alkaline or acid phosphatases. Morphological assessments of macrophage activity can also be made and these involve counting the number of pseudopodia and the number of phagocytic vacuoles present in the cell.

Macrophages are usually referred to as being 'stimulated' when increased activity is determined morphologically and as 'activated' when enzymatic techniques are used. 'Activated' or 'stimulated' macrophages have increased DNA levels, increased protein synthesis and an enhanced ability to kill bacteria such as Listeria monocytogenes. The technique used here to measure macrophage activity was the acid phosphatase test first described by Rosales, Bennett and Rutenburg (1966).

The second method of assessing macrophage activity was an inflammation test described by Normann and Schardt (1978). This test uses a standardised, biologically inert stimulus, a nitrocellulose filter, to elicit a subcutaneous inflammatory response and allows easy quantification of the cell types involved. Nitrocellulose filters do not produce the interstitial oedema that occurs when coverslips are used in similar tests. Included in the results described here are those obtained from the preliminary experiments in which the test was characterized prior to its use in N. dubius infected animals.

The section is completed by an investigation of the activity of lymphocytes from the mesenteric lymph node and spleen during N. dubius infection. The ability of cells from these organs to respond to stimulation by polyclonal T and B cell activators was examined in a macroculture system.

SECTION 1(2)

MATERIALS AND METHODS

ACID PHOSPHATASE ACTIVITY OF PERITONEAL MACROPHAGES

Acid phosphatase activity was assessed according to the method of Rosales, Bennett and Rutenburg (1966). Peritoneal exudate cells (PEC) were obtained from mice after i/p injection of 3ml of sterile medium 199. The medium was withdrawn and aliquoted into test-tubes containing small (14mm) round coverslips. Foetal calf serum was added to give a concentration of 10%. After six hours incubation (37°C) the coverslips were washed extensively to release non-adherent PEC. The adherent cells (macrophages) were air dried and fixed in 20% formalin in 50% acetone at 0°C for 1 minute, rinsed in tap water and air dried.

The fixed smears were incubated in a freshly prepared mixture of stock substrate solution and Fast Blue BBN. The stock solution was prepared by dissolving 30mg Naphthol AS phosphate (Sigma, Poole) in 0.25ml, N,N-dimethyl formamide (Sigma, Poole) and subsequently adding 100ml of acetate buffer pH 5.0. This stock solution is reported to be stable for several months (Rosales et al 1966). Before incubation 10mg of Fast Blue BBN was dissolved in 10ml of stock substrate solution and filtered rapidly.

After incubation smears were rinsed in tap water and air dried. Smears were counterstained with a 0.1% aqueous solution of Neutral Red for 2-3 minutes, air dried and mounted in DPX (BDH). Controls were one of three types:

1. smears incubated in diazonium salt without substrate (i.e. Naphthol AS Phosphate),

2. smears inactivated by immersion in boiling water for 1 minute,

3. smears incubated in stock substrate solution without diazonium salt (i.e. Fast Blue BBN).

Acid phosphatase activity appears as rod-like (or round) dark-blue cytoplasmic granules when smears are examined under oil immersion. Activity is measured by counting the number of granules per cell.

INFLAMMATION TEST

This technique was first described by Normann and Schardt (1978). Circular nitrocellulose filters (Sartorius Membrane Filters, V.A. Howe and Co. Ltd. London) measuring 13mm x 130μm and with a pore size of 0.2μm were selected for use in the test. At this pore size adherent cells are unable to penetrate into the interior of the filter.

Animals were anaesthetised as for laparotomy and lightly secured to the operating surface with adhesive tape, ventral side uppermost (see Materials and Methods section 2). An incision (1cm) was made in the skin, just right of the midline, and the subcutaneous tissue on the right of the incision separated from the body wall by means of blunt forceps. When a pocket had been formed a clean sterile filter moistened with sterile distilled water was inserted with dissecting forceps, 1-2cm from the skin incision. The sterile distilled water allows free manipulation of the filter by reducing adhesions. Once in place air was removed and the wound was closed with two sutures. On removal, filters were processed as shown below.

FIXING:-	96% ETHANOL	12 minutes
	70% ETHANOL	1 minute
STAINING:-	WEIGERTS IRON HAEMATOXYLIN	2 minutes
DEHYDRATION:-	70% ETHANOL (2 changes)	1 minute each
	95% ETHANOL (3 changes)	1 minute each
	80% ETHANOL/20% BUTANOL (3 changes)	1 minute each
CLEARING:-	XYLENE	45 minutes

Filters were then mounted in DPX on glass slides under coverslips. The inflammatory response was assessed by counting the cells in 10-20 randomly selected oil immersion fields.

POLYCLONAL ACTIVATORS

Two polyclonal activators were used: Phytohaemagglutinin (PHA, Wellcome), and Pokeweed mitogen (PWM, Gibco, Europe).

PHA is primarily a T-cell stimulator; PWM will stimulate both T and B lymphocytes and it triggers IgM synthesis and secretion in mouse B lymphocytes. The quantities of mitogens employed in the assays were determined by a dose response experiment (see Figure 34, 35).

MITOGEN STIMULATION ASSAY (MACROCULTURE SYSTEM)

Suspensions of spleen cells and mesenteric lymph node cells were prepared as for labelling (see Materials and Methods section 1) except that heparin was omitted from the supplemented RPMI 1640. Culture tubes were set up in sextuplet for each cell population with cells suspended at 10^6 /ml in 2ml of medium containing PHA or PWM at the required concentration. The tubes which had been gassed with 2% CO_2 prior to the addition of the cells, were incubated at

37 ± 0.5°C in a shaking water bath. Forty eight hours after the start of incubation 5-[^{125}I] Iodo-2'-deoxyuridine was added to give a final concentration of 0.1 Ci/ml. After a further 24 hours incubation, cells were washed thoroughly by centrifugation in label free medium. Labelling of cells was measured as described in Materials and Methods section 1(1). The degree of stimulation is given as an index:

$$\frac{\text{Counts /minute stimulated cells}}{\text{Counts /minute unstimulated cells}} = \text{stimulation index}$$

Stimulation indices are shown graphically (see Results 1(2)) with the range of control values represented by dotted lines.

SECTION 1(2)

RESULTS

MACROPHAGE ACTIVITY / MIGRATION

Having dealt with the complexities of the cell transfer system and gained some, but not enough information on the mechanism of immunosuppression which operates during N. dubius infections an examination of macrophage and lymphocyte activity during infection was undertaken in order to determine if the effect of N. dubius was to interfere with the activities of these cell types.

The first experiments were designed to assess the acid-phosphatase content of macrophages as an index of macrophage activity and used the technique described in the materials and methods section. Before applying the technique to N. dubius infected mice acid-phosphatase activity was measured in mice which had been injected (i/p) with the bacterium, Corynebacterium parvum which is known to activate macrophages.

In the first experiment 3 male NIH mice (6 weeks old) were injected i/p with 0.18ml (0.7mg) of a suspension of C. parvum, obtained from the Department of Immunology, University of Edinburgh. Three days after this these mice plus a group of control, uninjected mice were killed and peritoneal exudate macrophages were harvested and processed. On examination it was impossible to distinguish acid-phosphate activity from ingested bacteria. There appeared to be more acid phosphatase activity in the C. parvum-treated group but this may have been the result of the staining of the bacterium. An experiment identical to this in which mice were killed on day 8 after injection of C. parvum gave a similar result although the difference was not as marked.

In the final experiment groups of 4 mice were treated in the following ways. The first group were each given 0.18ml (0.7mg) C. parvum (i/p), the second group were given 300 N. dubius and the third remained as uninfected controls. These mice were killed on day 3 and peritoneal macrophages were harvested for assessment of acid-phosphatase activity. On this occasion the test was run for 1 hour and six hours. The levels of acid-phosphatase activity recorded did not vary between groups at either time although the activity was greater at six hours. It must be emphasised that these were not quantitative assessments, since the amount of activity in each cell was too great to be counted accurately. Although this method has some advantages over other techniques of measuring enzyme activity in macrophages, it was not possible using it to quantify differences despite the optimistic comments of the authors who described the technique.

The second attempt to measure macrophage activity employed the macrophage inflammation test. Although this test measures numbers of macrophages on a nitro-cellulose filter rather than the enzyme activity in individual macrophages it would seem not unreasonable to assume that a macrophage which is 'activated' or 'stimulated' (see Introduction to this section) will in fact be more motile and more responsive to stimuli. The results are interpreted to allow for this limitation.

In the first experiment (Experiment 18) nitro-cellulose filters were implanted into 24, 6 week old NIH mice. Immediately before implantation 12 of these mice were infected with 300 N. dubius. A careful note was taken of the time of implantation to allow mice to be killed at the specified times. Animals were killed 2/group at 24, 48, 72, 96 and 216 hours after implantation, the filters were

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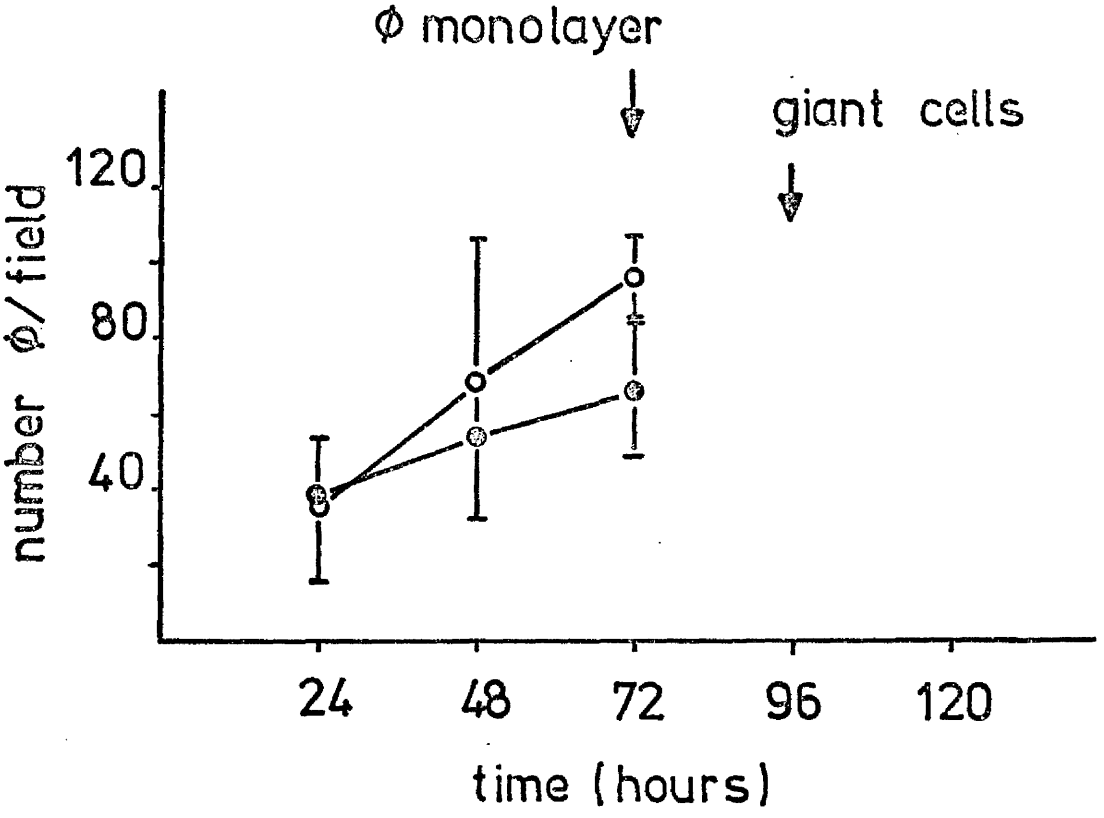
FIGURE 29

Inflammation test

Accumulation of macrophages on s/c implanted filters at 24, 48, 72 and 96 hours.
(number ø / field ± SD)

● *N. dubius* infected mice

○ Control, uninfected mice


Ø monolayer
giant cells
number Ø/field
120
80
40
24
48
72
96
120
time (hours)

removed, prepared and counted as described (Materials and Mehtods). The results are shown in Figure 29.

By 24 hours macrophages had begun to accumulate on the filters. There were a few other cell types present on the filter at this time notably eosinophils and a number of neutrophils. These cell types, usually the cell types associated with acute inflammatory responses had disappeared from the filters by 48 hours post implantation and at this time the number of macrophages on the filters had increased. The increase in numbers continued and a complete monolayer of macrophages was formed by 72 hours. By 96 hours Foreign body and Langhans Giant cells were visible and it became impossible to make an accurate count of the numbers of cells present on the filter. These epithelioid cell types (so called because they resemble epithelial cells) show a large increase in the amount of cytoplasm they possess, and are not actively phagocytic. They are multinucleate cells which are formed by the fusion of individual macrophages. When the cells on the filter had reached this stage counting was stopped.

As far as the cells on the filters in N. dubius infected mice were concerned the initial 24 hour count was identical to that of the control group. At 48 hours the numbers of cells had increased but not to the same extent as on filters from the control group and this pattern was repeated at 72 hours. From the results it seems that the rate of accumulation of the cells on the filters in N. dubius infected mice was slower, but again by 96 hours Giant cell formation had started so it would appear that the macrophage responses were similar in both groups over this early period of infection.

The first experiment (Experiment 18) provided the basic information that was needed before using the filters in a larger experimental programme. In the next experiment (Experiment 19) filters were implanted for 72 hour periods in both control and in infected mice. On day 0, 28 male NIH mice were infected with 300 N. dubius and six were left uninfected as controls.

On each day of infection two mice had filters implanted. Control mice (2/group) had filters implanted on days 0, 7 and 13. All filters were removed 72 hours later. The results are shown in Figure 30. Macrophage counts are plotted against the day of filter implantation. There was some variation in the macrophage counts obtained in this experiment but on the whole the counts from infected mice distributed themselves evenly around the mean values obtained from the control (uninfected) mice on days 0, 7 and 13. The values obtained for control and infected macrophage numbers were not as high as those obtained in the previous experiment. The conclusion from this experiment must be that there is no difference in the macrophage response to filters in control and infected animals.

This experiment was repeated with controls and infected mice both having filters implanted on days 0, 2, 4, 6, 8, 12, 14 and 16. Mice were 6-8 week old NIH and were killed in groups of 2. Filters were left in situ for a 72 hour period. The results are shown in Figure 31 (Experiment 20).

On this occasion macrophage numbers were very high early in the experiment in both infected and control animals. The numbers dropped as the experiment continued but no statistically significant differences were ever observed.

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FIGURE 30

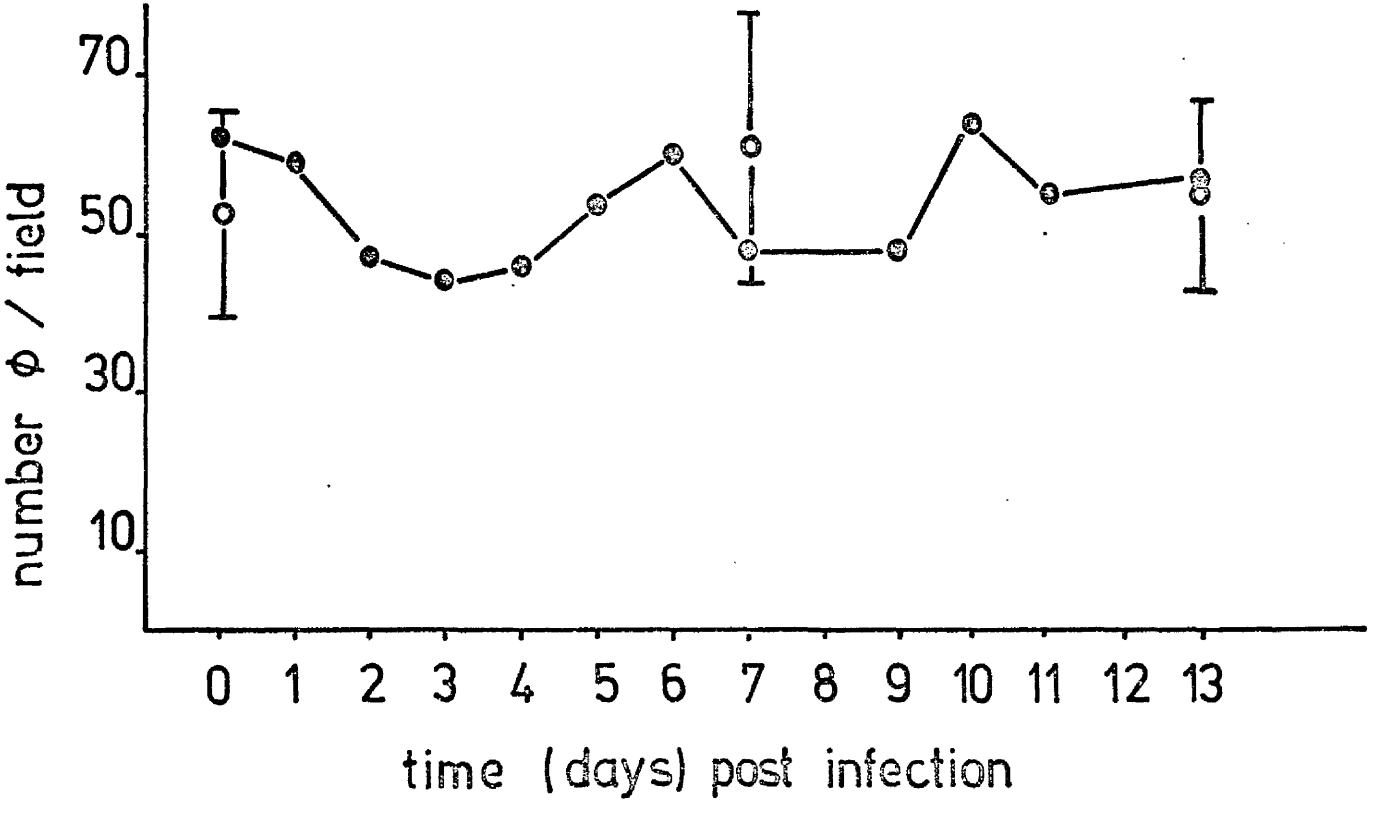
Inflammation test

Accumulation of macrophages on s/c implanted filters at 72 hours (number ø /field ± SD)

Filters implanted at various times after infection with N. dubius and results plotted against the day of filter implantation

● N. dubius infected mice

○ Control, uninfected mice


70
50
30
10
number ϕ / field
0 1 2 3 4 5 6 7 8 9 10 11 12 13
time (days) post infection

Facing page 93

FIGURE 31

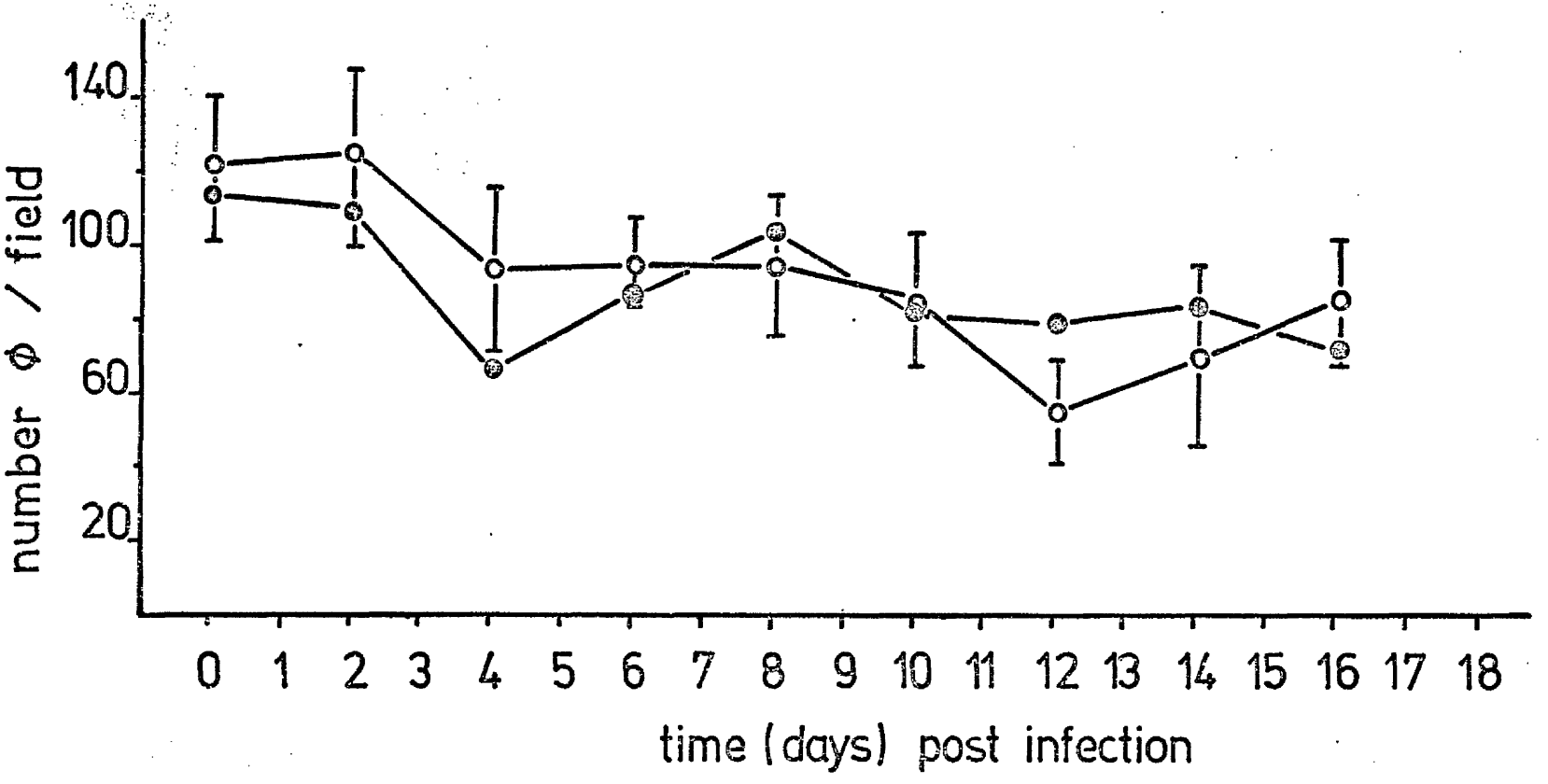
Inflammation test

Accumulation of macrophages on s/c implanted filters at 72 hours (number ø /field ± SD)

Filters implanted at various times after infection with N. dubius and results plotted against the day of filter implantation

● N. dubius infected mice

○ Control, uninfected mice


140
100
60
20
number ϕ / field
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
time (days) post infection

Since the only difference detected in all the experiments using the filters was a slight difference in the rate of accumulation of cells on the filters in the first experiment of the series (Experiment 18), it was decided to examine the rate of accumulation within the first 24 hours of infection, since the early accumulation may be the critical step in the process of inflammation and may be more susceptible to the interference of N. dubius.

Ten mice were infected with 300 N. dubius and immediately afterwards these, plus ten uninfected mice had filters implanted. Filters were removed for fixing, staining and counting 6, 12, 16, 20 and 24 hours after implantation. The results for this experiment (Experiment 21) are shown in Figure 32.

The number of polymorphs on the filters was very high even after only 6 hours implantation but then dropped steadily over the next eighteen hours to reach low levels in control and infected mice. The macrophages followed the opposite pattern. Only 1 or 2 macrophages were present in any field early in the response (see Figure 32, 6 and 12 hours). After twelve hours the numbers of macrophages began to increase reaching about 50/field in both control and infected animals by 24 hours.

To complement the previous experiment a study of the six hour response at various times during infection was also completed. Eighteen male mice were infected with 300 N. dubius and 18 were retained as uninfected controls. Two mice from each group had filters implanted on days 0, 2, 4, 6, 8, 10, 12, 14 and 16 of infection and these filters were removed six hours after implantation. The results are shown in Figure 33 (Experiment 22).

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FIGURE 32

Inflammation test

Accumulation of macrophages and polymorphs on s/c implanted filters at 6, 12, 16, 20 and 24 hours
(number ∅ /field or number polymorphs/field)
(SD not shown for clarity).

● ■ N. dubius infected mice

○ □ Control, uninfected mice

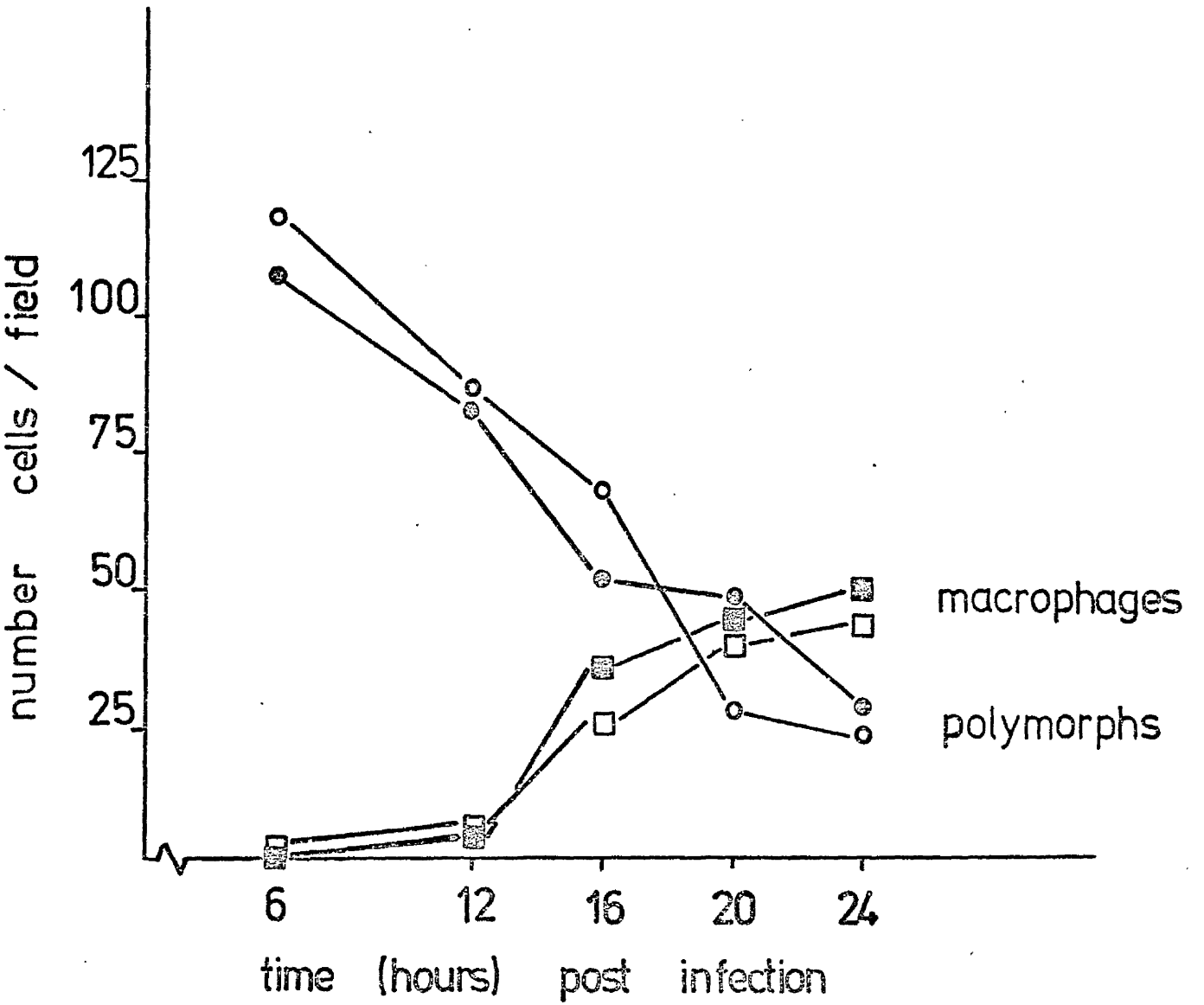
number cells / field
125
100
75
50
25
6
12
16
20
24
time (hours) post infection
macrophages
polymorphs

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FIGURE 33

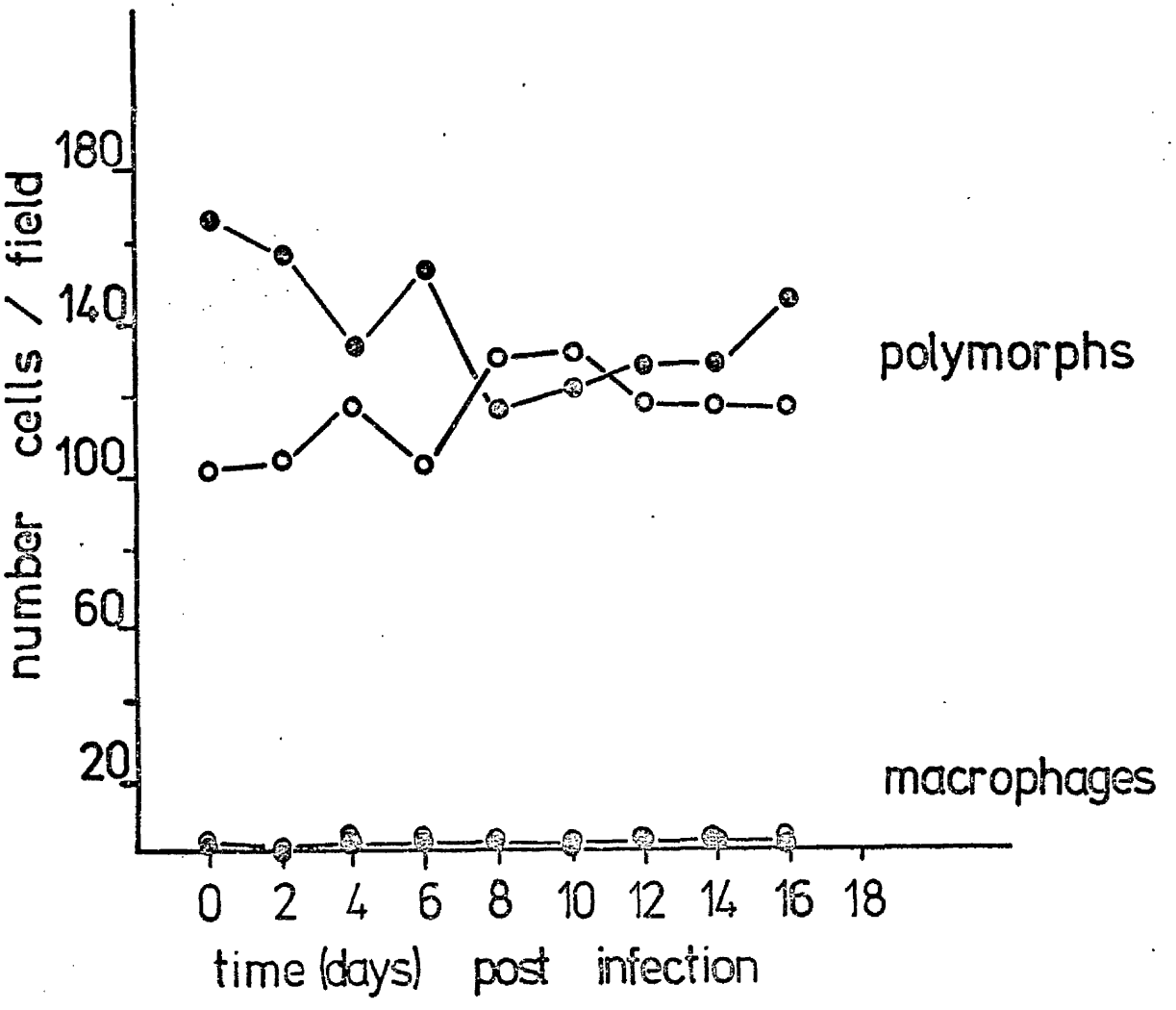
Inflammation test

Accumulation of macrophages and polymorphs on s/c implanted filters at 6 hours
(numbers ø /field or number polymorphs/field)
(SD not shown for clarity).

Filters implanted at various times after infection with N. dubius and results plotted against the day of filter implantation

● N. dubius infected mice

○ Control, uninfected mice


number cells / field
180
140
100
60
20
polymorphs
macrophages
0 2 4 6 8 10 12 14 16 18
time (days) post infection

As was shown in the previous experiment (Experiment 21) the macrophage response at 6 hours was very low. This was consistent for both control and infected mice throughout the experiment. The early cell infiltration is dominated by polymorphonuclear leukocytes, predominantly neutrophils and eosinophils but with a few tissue mast cells. There was also evidence, at this stage, for a good deal of fibroblast activity particularly towards the edges of the filters. One problem with filters taken at this early stage after implantation is that the cell types on the filter are less adherent and tend to strip off in sheets, folding back on themselves to give a multilayered effect. Extreme care is required when removing and processing these filters. As a result the counts in this 6 hour response experiment are based on 10 oil immersion fields rather than the usual 20. The important point in this experiment is that numbers of macrophages in infected and control animals were similar throughout the whole period of the experiment. The polymorph numbers were essentially the same in both groups except for the first 6 days when there was an increased number in infected animals. This difference was not maintained.

One other technique to assess macrophage activity was attempted. This was a cytoxicity test in which macrophages (peritoneal exudate) were cultured with labelled tumour cells (B16 Melanoma) but the results were highly variable in two preliminary experiments and as a result the test was abandoned.

LYMPHOCYTE RESPONSIVENESS

PHA AND PWM STIMULATION

Before using these tests to measure lymphocyte responsiveness an experiment to determine the dose response curve was completed.

The results are shown for PHA in Figure 34 and for PWM in Figure 35. The results for PWM were unexpected in that despite the range of doses used the response was a linear plateau rather than a smooth curve. Either this was the peak stimulation value possible in our system or the amount of stimulation required to obtain peak values was not present. PWM was not included in later experiments. PHA on the other hand gave a clear response curve and in later experiments a dose of 1.0μg/ml of culture medium was employed. During the course of the mitogen experiments the scintillation counter was readjusted and as a result the readings were greatly increased. In order to present uniform results therefore, a stimulation index is given in place of an absolute count.

In the first experiment (Experiment 23) MLNC and SC were taken from male NIH mice which had been infected with 300 N. dubius 4, 8 and 12 days previously. Cells from control, uninfected animals were also tested. Background isotope incorporation levels were determined by culturing cells in the absence of mitogen. The results are shown in Figure 36. The dotted lines represent the range of control values. Spleen cells were tested only on day 4 of infection.

The stimulation indices shown are lower than those obtained whilst determining the dose-response curve, however, such variability must be expected in a system such as this. The results show that on day 8 of infection the MLNC from N. dubius infected mice failed to respond to PHA stimulation and in fact in terms of absolute counts had lower isotope incorporation than their unstimulated controls. Cells on days 4 and 12 responded to PHA and gave indices of 6-9.

Two more experiments in which responsiveness was assessed on days 16, 20 and 25 (Experiment 25) of infection were carried out.

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FIGURE 34

Mitogen stimulation of MLNC

Stimulation index at 0, 0.5, 1.0 and 2.5 µg PHA/ml.

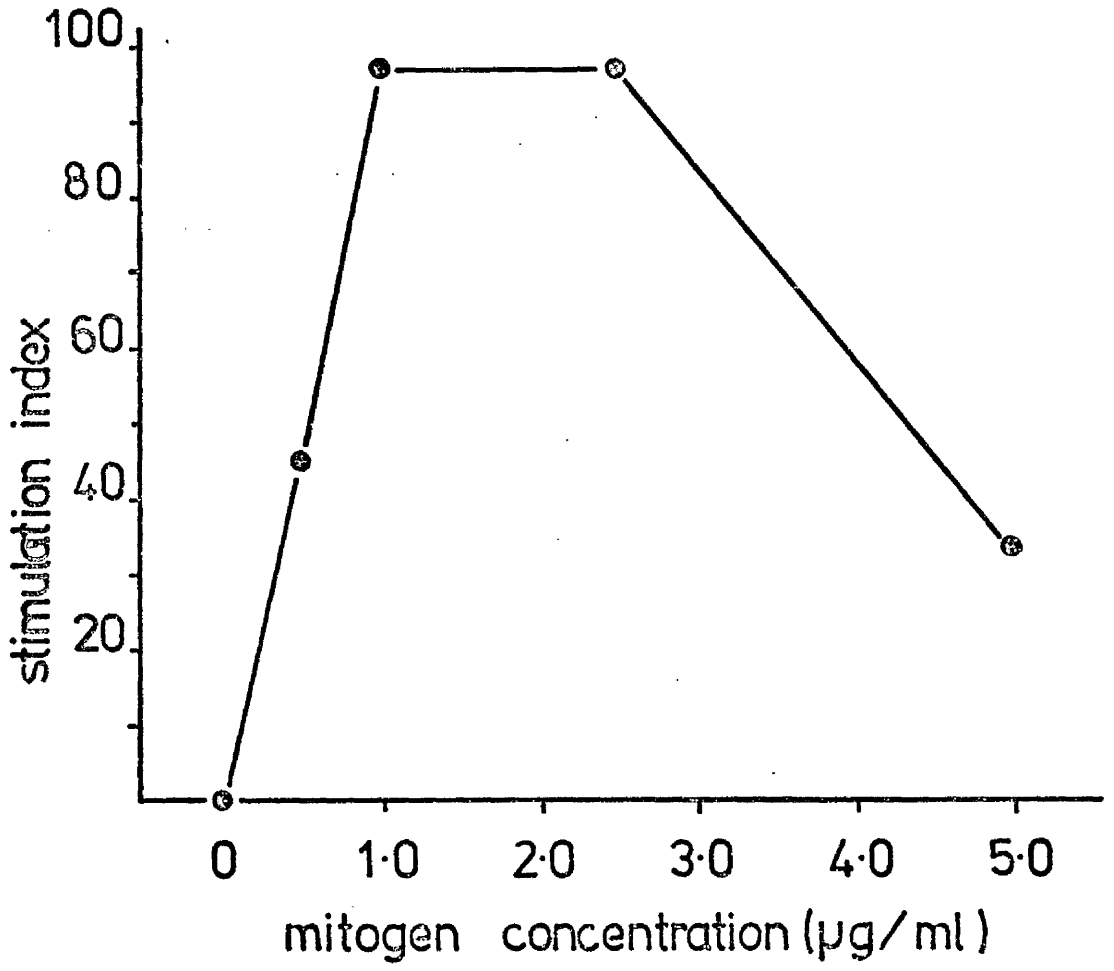
100
80
60
40
20
stimulation index
0
1·0
2·0
3·0
4·0
5·0
mitogen concentration (µg/ml)

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FIGURE 35

Mitogen stimulation of MLNC

Stimulation index at 0, 0.5, 1.0, 2.5, 5.0 and 10 µl PWM/ml

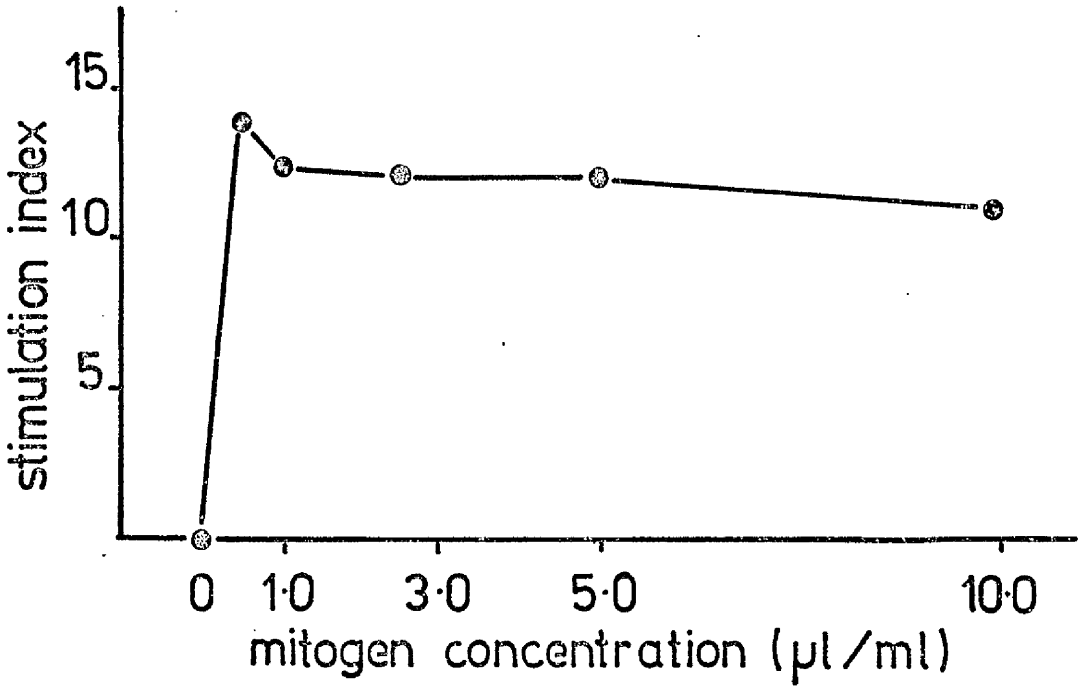
stimulation index
15
10
5
0
1·0
3·0
5·0
10·0
mitogen concentration (µl/ml)

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FIGURE 36

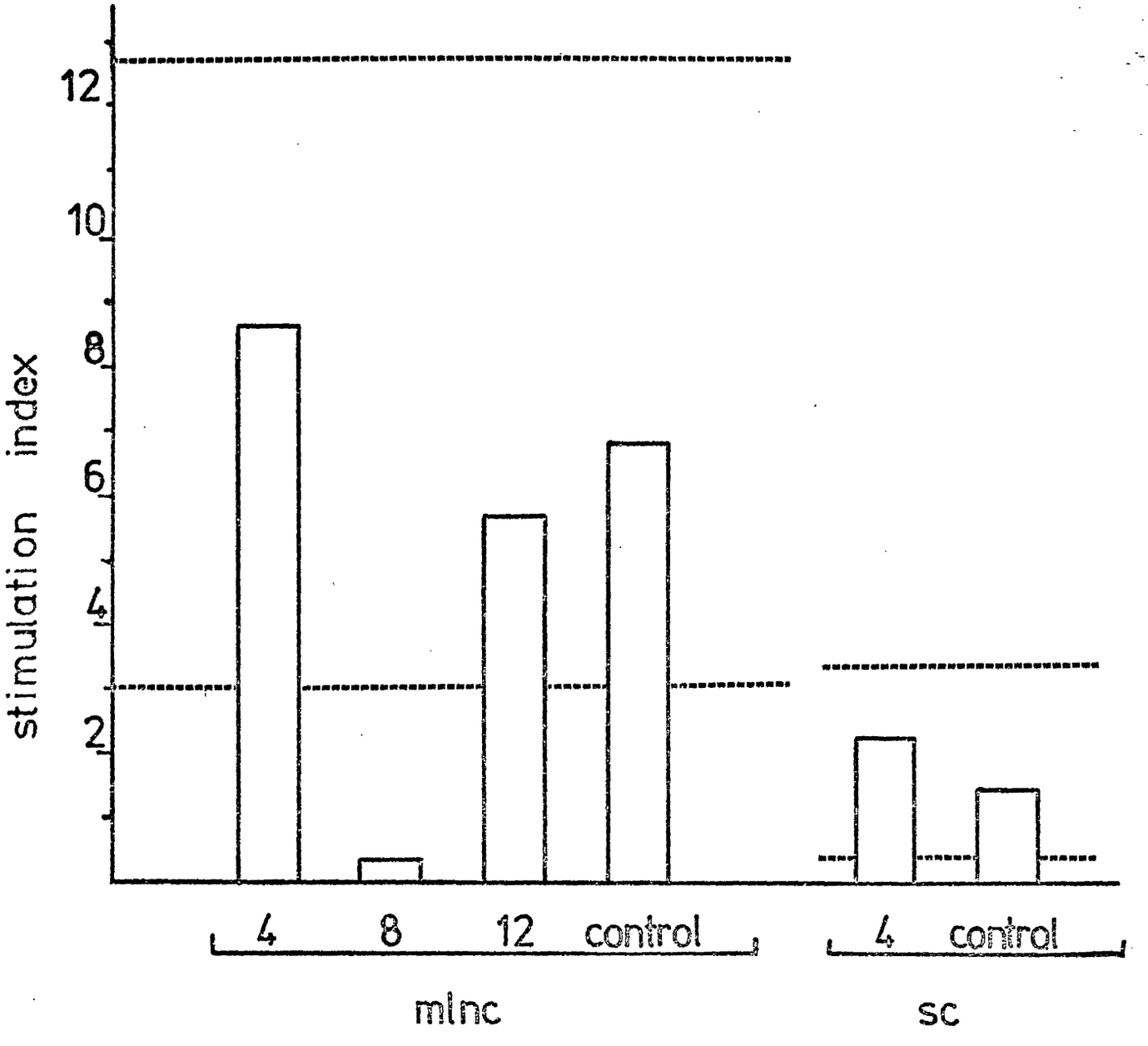
Mitogen stimulation of MLNC and SC

(1 µg PHA/ml culture)

Stimulation index on days 4, 8 and 12 of *N. dubius* infection.

Control, uninfected mice

Error bars represent range of control values


stimulation index
12
10
8
6
4
2
4
8
12
control
4
control
mlnc
sc

Cells from uninfected mice were tested on both these days and spleen cells with unstimulated controls were tested on days 16 and 40 only. Infected mice were given 300 N. dubius. The results are shown in Figures 37 and 38. MLNC from infected mice did not respond as well as did MLNC from uninfected mice at any of the times shown in these two figures. The SC responsiveness was not as good as that of MLNC on days 4 (Figure 36), 16 (Figure 37) and 40 (Figure 38) and showed a low level of stimulation throughout. The reason for this may be that the PHA dose response curve was determined using MLNC and not SC which may have different requirements, particularly with regard to mitogen concentration, for good stimulation. The results so far have indicated that the major change in MLNC reactivity to mitogenic stimulation by PHA occurs at day 8 of infection although responsiveness was depressed throughout the first 40 days in infected animals.

To complete this section on mitogen responsiveness an experiment involving concurrent N. dubius and T. spiralis infections was set up. In this experiment groups of 6 NIH mice were infected with N. dubius and/or T. spiralis with another group retained as uninfected controls. A mitogen assay was set up using the day 8 MLNC from these groups as shown below:

A 300 N. dubius MLNC (day 8)

B 300 T. spiralis MLNC (day 8)

C 300 N. dubius MLNC (day 8) x 300 T. spiralis MLNC (day 8) concurrent infection.

D 300 N. dubius MLNC (day 8) + 300 T. spiralis MLNC (day 8) mixed cells.

E Control cells from uninfected mice.

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FIGURE 37

Mitogen stimulation of MLNC and SC

(1μg PHA/ml culture)

Stimulation index on days 16, 20 and 25 of *N. dubius* infection

Control, uninfected mice

Error bars represent range of control values.

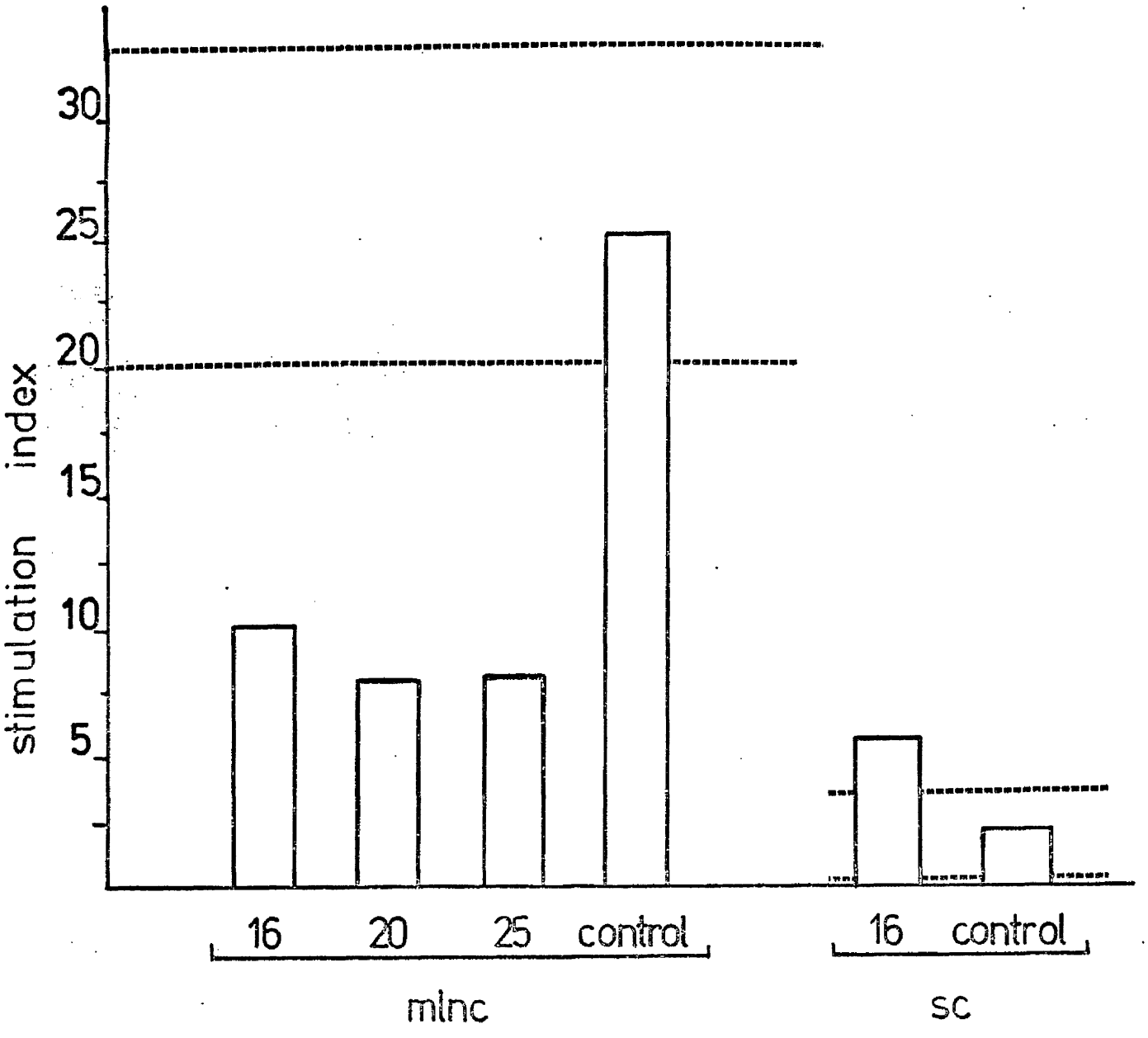
stimulation index
30
25
20
15
10
5
16
20
25
control
16
control
mlnc
sc

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FIGURE 38

Mitogen stimulation of MLNC and SC

(1μg PHA/ml culture)

Stimulation index on days 30, 35 and 40 of *N. dubius* infection

Control, uninfected mice

Error bars represent range of control values

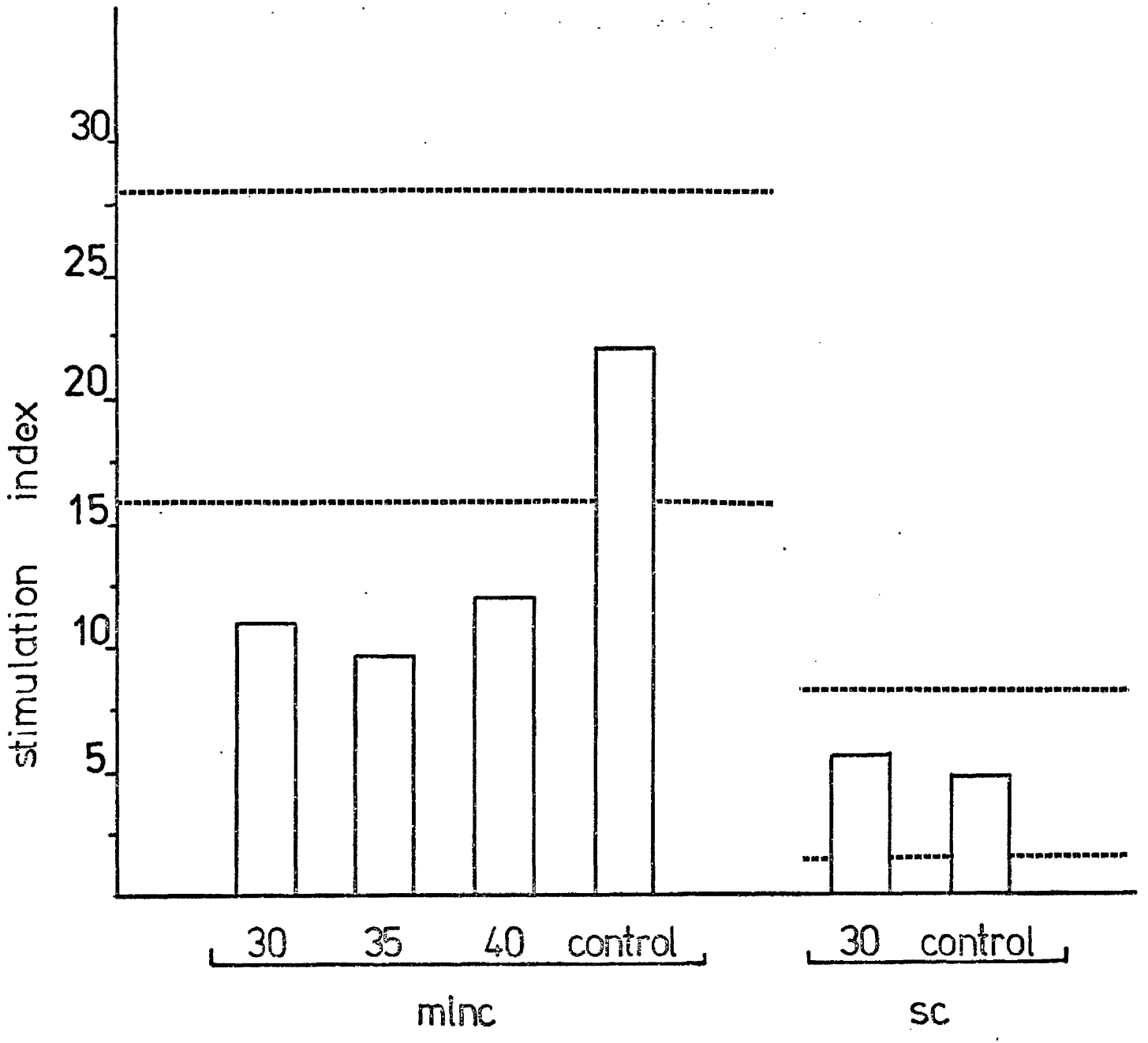
stimulation index
30
25
20
15
10
5
30
35
40
control
30
control
minc
sc

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FIGURE 39

Mitogen stimulation of MLNC

(1µg PHA/ml culture)

Stimulation index

GROUP

A 300 *N. dubius* MLNC (day 8)

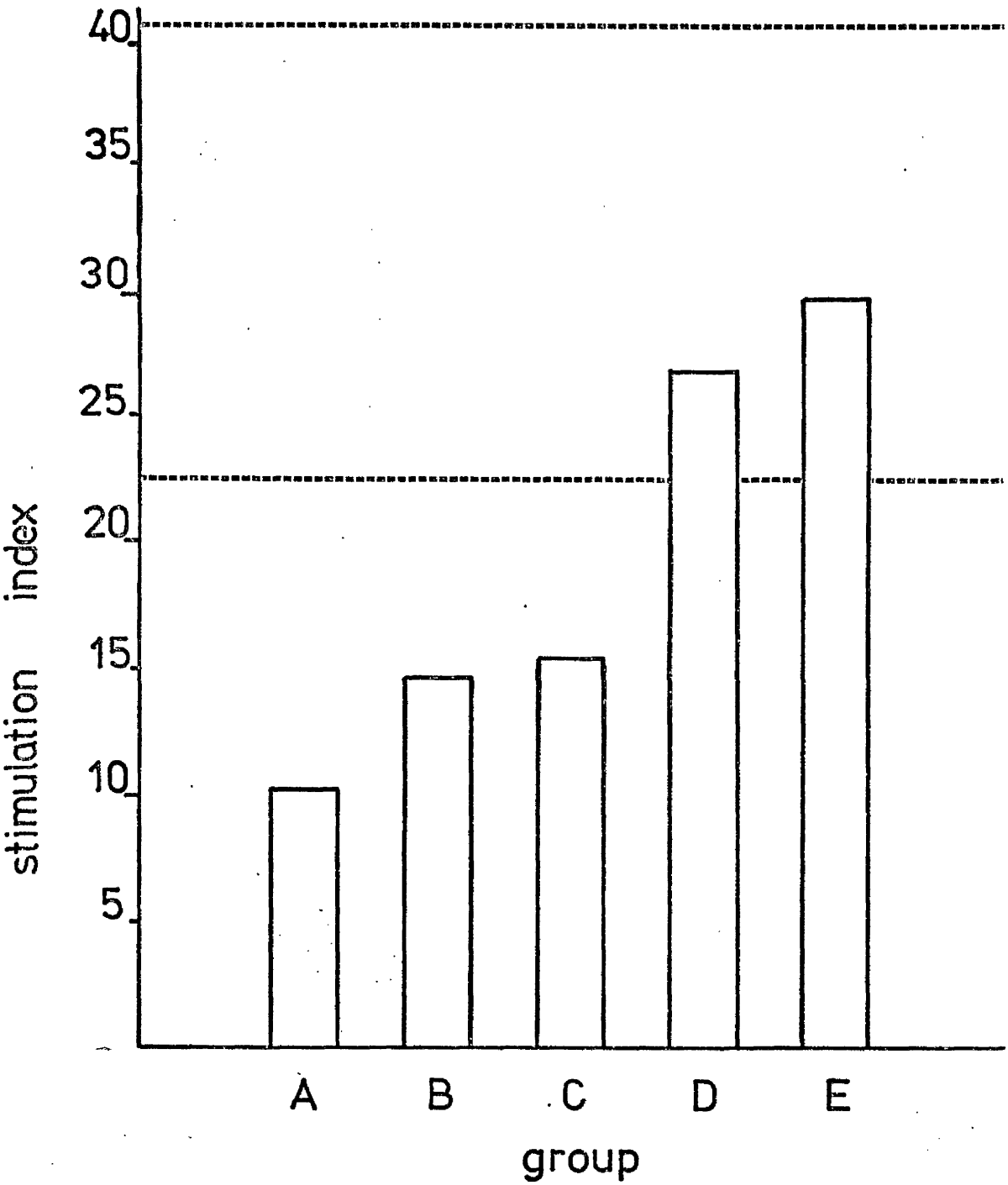
B 300 *T. spiralis* MLNC (day 8)

C 300 *N. dubius* MLNC (day 8) x 300 *T. spiralis* MLNC (day 8) concurrent infection

D 300 *N. dubius* MLNC (day 8) + 300 *T. spiralis* MLNC (day 8) mixed cells

E Control cells from uninfected mice

Error bars represent range of control values.


40
35
30
25
20
15
10
5
stimulation index
A
B
C
D
E
group

Unstimulated MLNC were again used as background isotope incorporation controls. The object of including the mixed cells group (Group D), was to determine if the cells from N. dubius infected mice had any effect on the responsiveness of cells from T. spiralis infected mice. The results (Experiment 26) are shown in Figure 39. Responsiveness in terms of stimulation indices was down in the N. dubius and T. spiralis single infections and in the N. dubius / T. spiralis concurrent infection, relative to control cells. An unexpected result was obtained from the mixed cells which gave control stimulation levels even though the separated cells were poorly responsive.

'IN VIVO' BLASTOGENESIS

This section of the work was carried out in conjunction with the 'in vitro' mitogen stimulation assay. The first part of the work involved counting the number of cells present in the MLN of infected and control animals and assessing the relative numbers of T and B cells which were present. The second part examined the in vivo cell division in the MLN, PP, spleen, small intestine and axillary lymph node measured by the level of ^{125}I-Udr incorpoation in the first two hours after injection.

For the cell counts and T and B proportions in the MLN, 36 male NIH mice were infected with 300 N. dubius and 36 were retained as uninfected controls. On days 4, 8, 12, 16, 20, 25, 30, 35 and 40 four uninfected mice were killed and the MLN processed individually. Cell counts and MLN weights (means of 4) are shown in Figure 40 (Experiment 27). The proportions of T:B cells remained consistent throughout the experiment and are not illustrated. MLNC numbers were low early in infection and rose steadily to remain at high levels. At this early stage of infection the larval stage of the parasite is situated in the muscularis externa and the low number of cells in

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FIGURE 40

Numbers of cells and weight of the mesenteric lymph node at various times after *N. dubius* infection

● ■ *N. dubius* infected mice

○ □ Control, uninfected mice

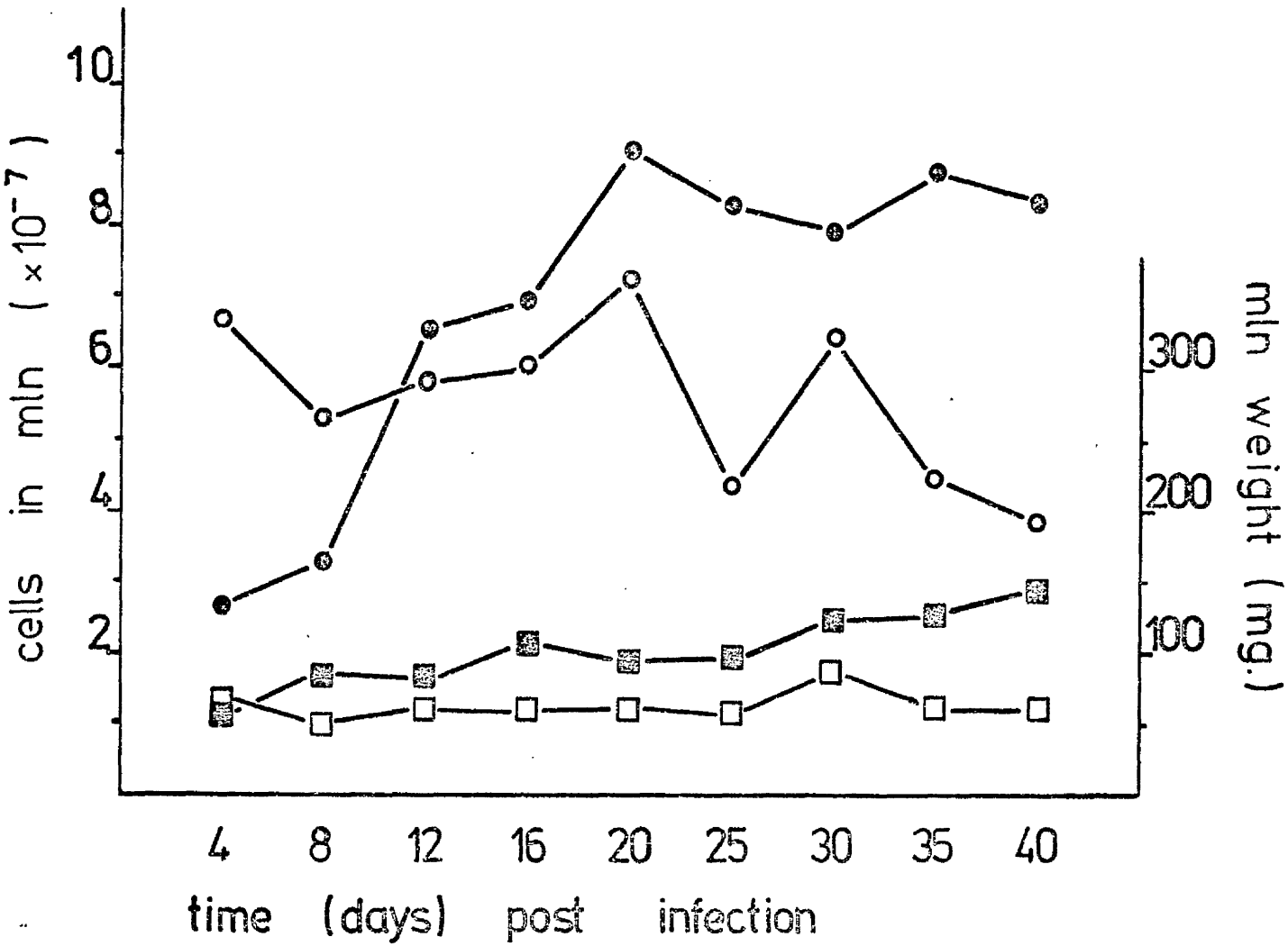
cells in mln (x10⁻⁷)
10
8
6
4
2
mln weight (mg.)
300
200
100
4
8
12
16
20
25
30
35
40
time (days) post infection

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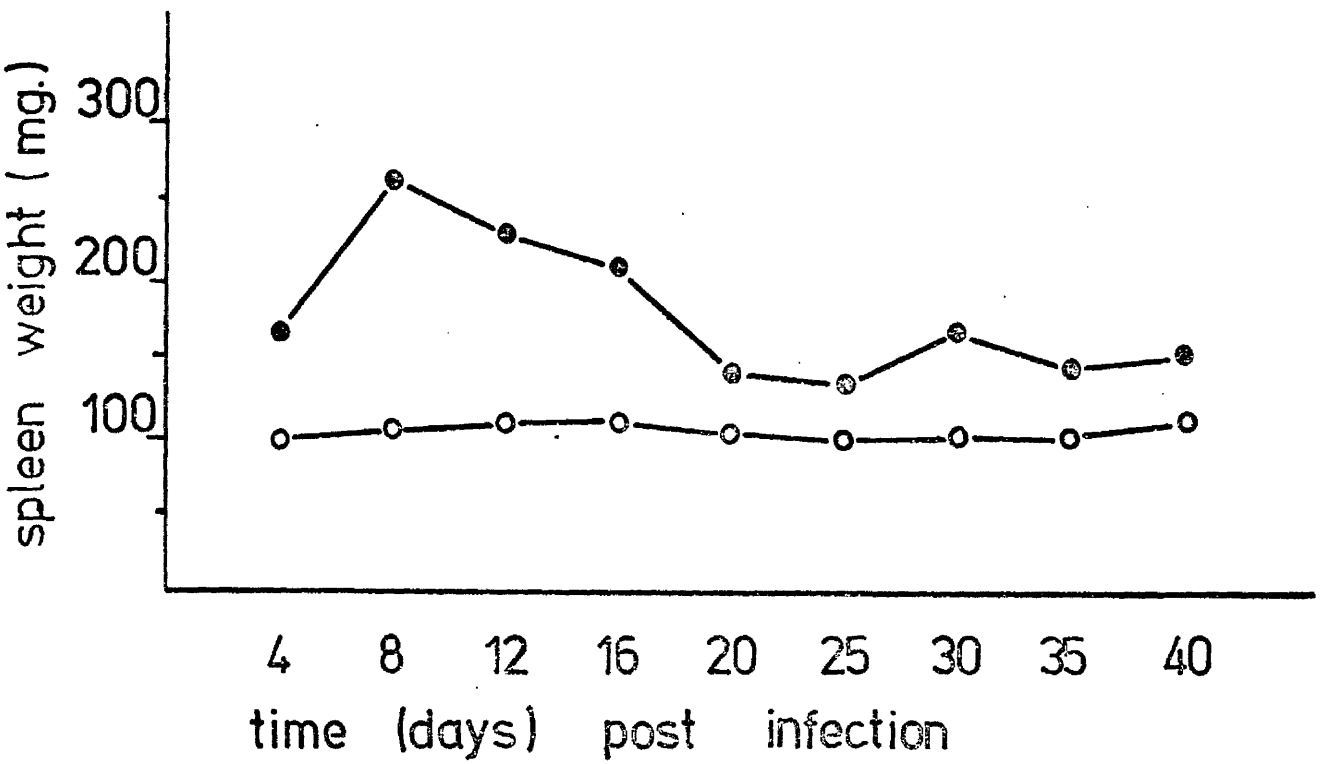
FIGURE 41

Weight of the spleen at various times after *N. dubius* infection

- ● *N. dubius* infected mice
- ○ Control, uninfected mice


spleen weight (mg.)
300
200
100
4
8
12
16
20
25
30
35
40
time (days) post infection

the MLN may have been due to a movement of cells from the node to the tissue surrounding the parasite. In some of the early cell transfer experiments recorded earlier, cell donors infected with N. dubius also on occasion gave very low cell recoveries. Spleen weights are shown in Figure 41 (Experiment 27). An increase in spleen size, reflected in the weights is apparent in the infected mice starting on day 4. Those rose to a peak level by day 8 and remained above control levels.

'IN VIVO' LABELLING

Thirty six male NIH mice were infected with 300 N. dubius on day 0 and these plus 36 uninfected age matched controls were killed on days 4, 8, 12, 16, 20, 25, 30, 35 and 40, 2 hours after injection with ^{125}I-Udr. Organs and tissues were removed, counted and dried before weighing. Results (Experiment 28) are expressed as Total count per organ and as count per mg. dry weight of organ (see Figures 42 and 43). Counts for the axillary lymph node are excluded as these were never above background counts. The counts for the other organs remained basically the same in both control and infected animals with the exception of the spleen. The total organ counts for the spleen started to diverge on day 8 in the infected mice. The counts rose until day 12, fell slightly on day 16 and remained at a raised level, approximately double control value until the end of the experiment. On an activity/mg. dry weight basis it was impossible to pick up differences in any of the organs.

EXCRETORY-SECRETORY PRODUCTS AND LYMPHOCYTE VIABILITY

This section has examined the immunosuppressive effect of N. dubius in the mouse but has not examined the ways in which the parasite causes this effect. One possibility is that the parasite produces an ES product which has a direct effect on mouse lymphoid

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FIGURE 42

'In vivo' labelling

Total counts/organ (individual samples plotted)

2 hours after injection with ^{125}I-Udr at various times during *N. dubius* infection

● *N. dubius* infected mice

○ Control, uninfected mice

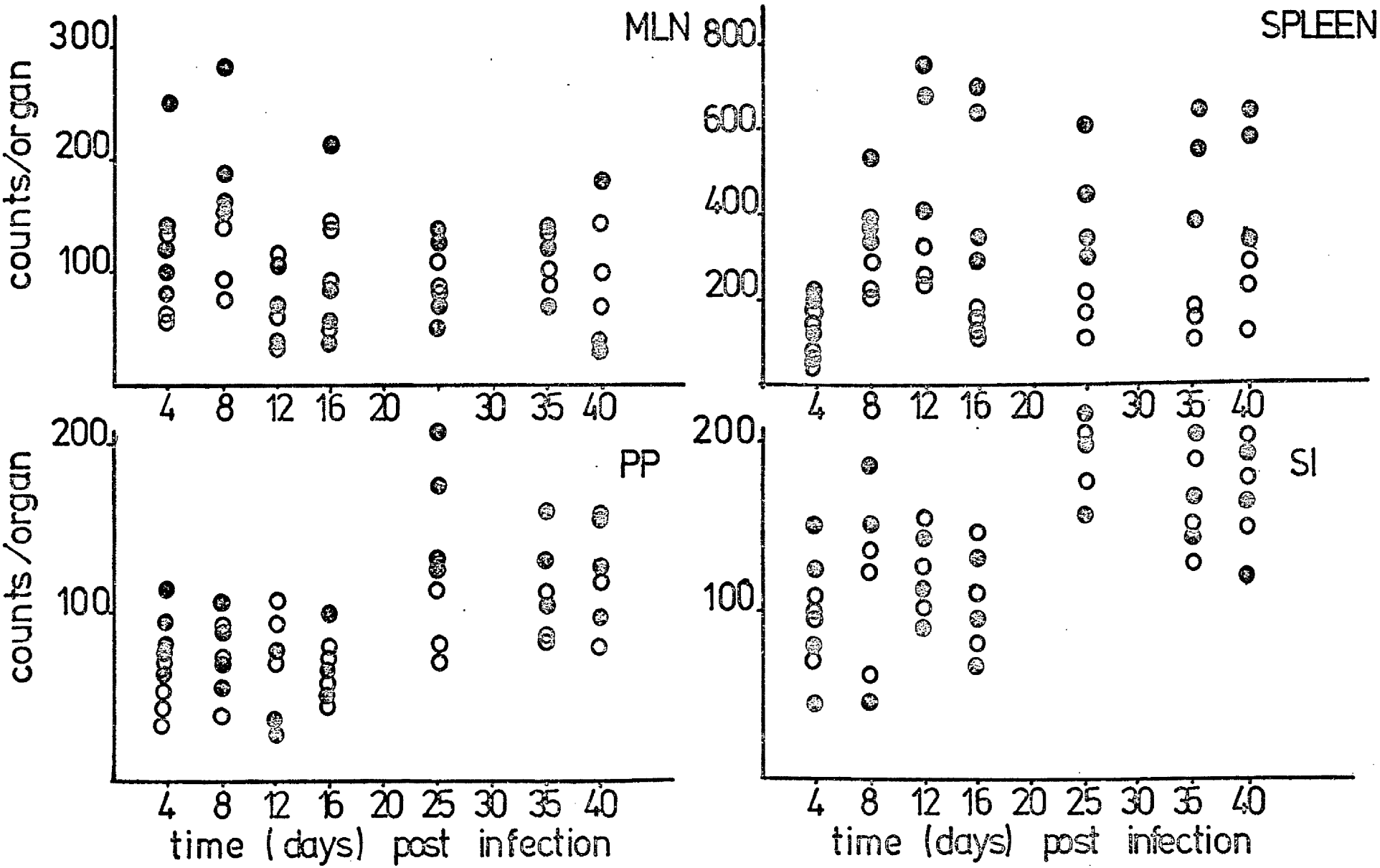
MLN
SPLEEN
PP
SI
counts/organ
counts/organ
300
200
100
800
600
400
200
200
100
4 8 12 16 20 30 35 40
4 8 12 16 20 25 30 35 40
time (days) post infection
time (days) post infection

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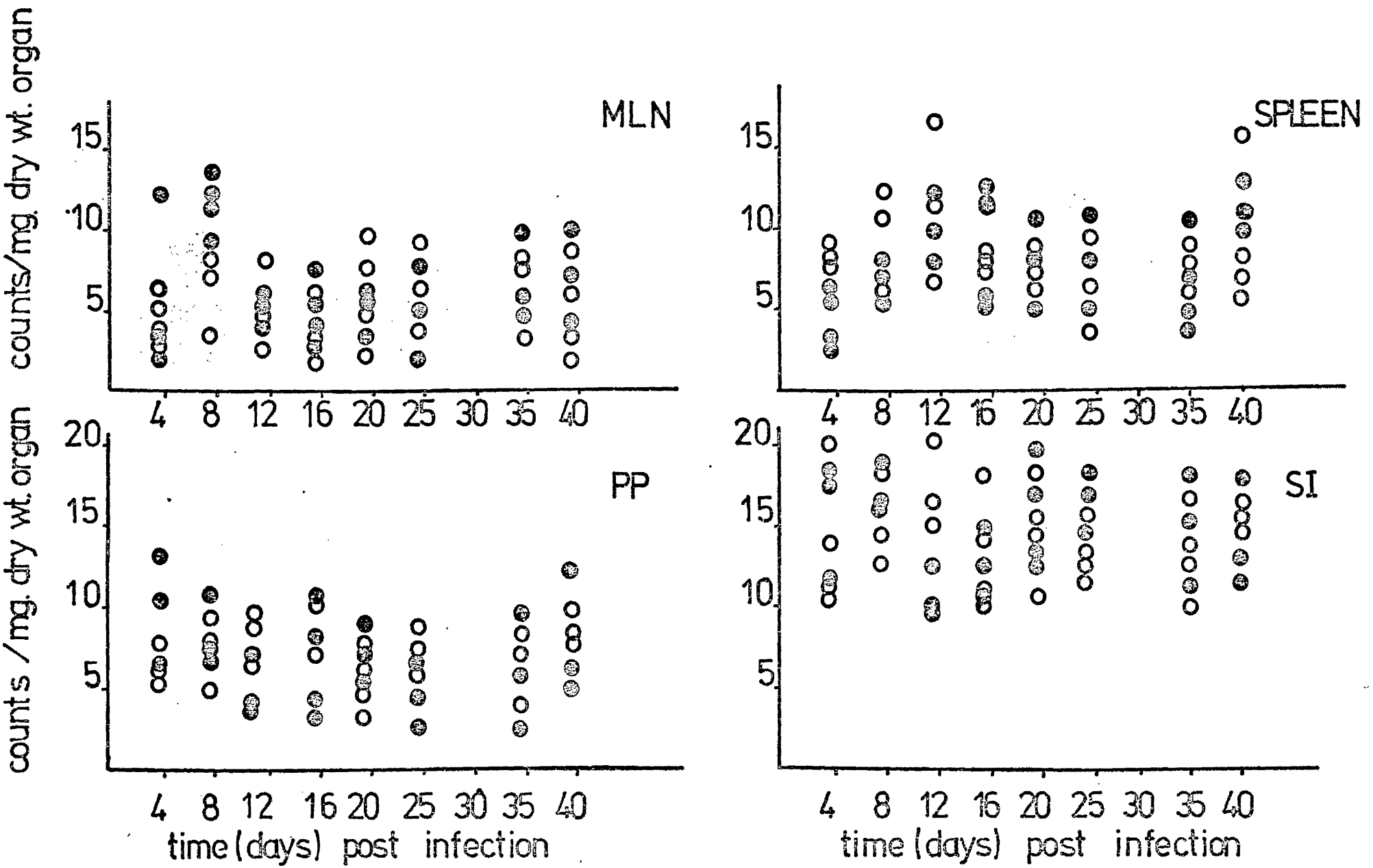
FIGURE 43

'In vivo' labelling

Counts/mg dry weight organ (individual samples plotted) 2 hours after injection with ^{125}I-Udr at various times during *N. dubius* infection

● *N. dubius* infected mice

○ Control, uninfected mice


MLN
SPLEEN
PP
SI
counts/mg. dry wt. organ
counts /mg. dry wt. organ
15
10
5
20
4 8 12 16 20 25 30 35 40
time (days) post infection

cells. To examine this ES products of N. dubius adult worms were collected and concentrated (x10) using an Amicon Filtration apparatus (cut off point m.w. 10,000). The concentrated ES products were added to MLNC cultures, control cultures being given an equivalent volume of medium 199, and cell viability was assessed at intervals over the following 2 hours. The results, two experiments combined are shown in Figure 44, Experiment 29. There was no evidence of a direct cytotoxic effect of N. dubius ES products on mouse MLNC. Although this is perhaps not the most effective way of measuring such effects, the concentration used in these experiments was much higher than would ever be experienced by MLNC 'in vivo'.

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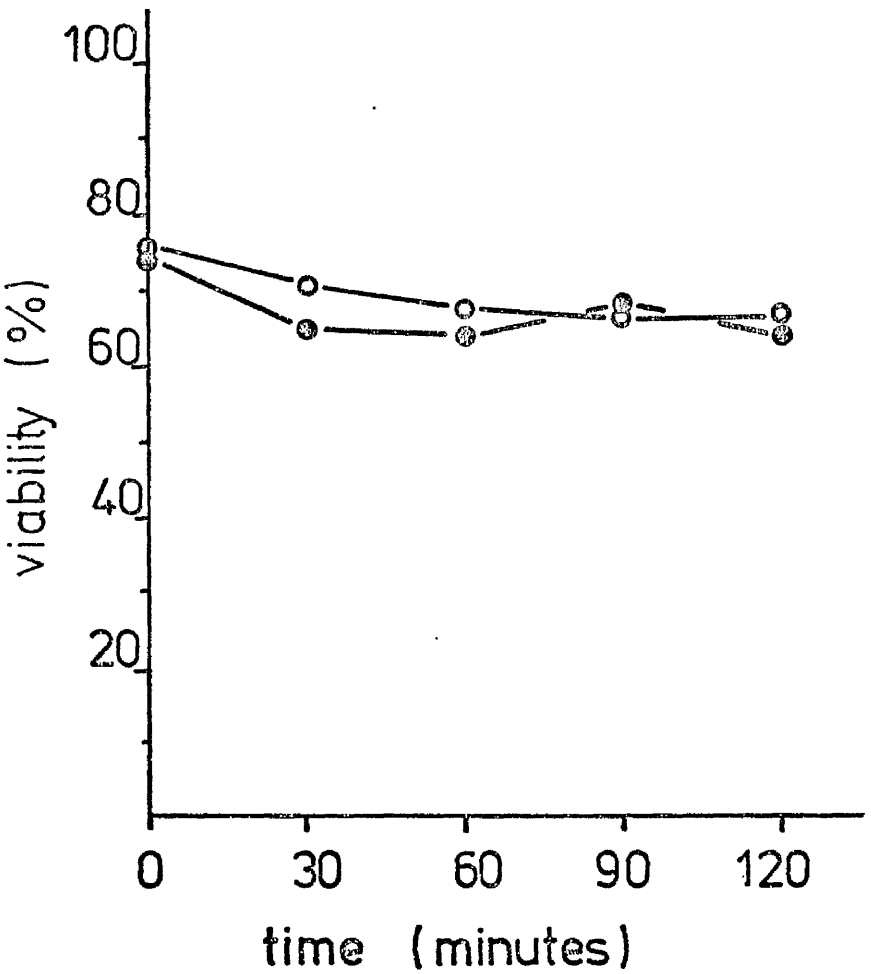
FIGURE 44

Effect of N. dubius ES-products (concentrated x 10) on MLNC viability.


100
80
60
40
20
viability (%)
0
30
60
90
120
time (minutes)

DISCUSSION

The results presented in Sections 1(1) and 1(2) have shown that *N. dubius* has a marked suppressive effect on the immune responsiveness of mice. The first two experiments confirmed the work of Behnke *et al.* (1978) that the immune expulsion of *T. spiralis* from NIH mice is delayed during concurrent infection with *N. dubius*. It was not merely the expression of immunity to *T. spiralis* which was impaired as the ability to transfer immunity to *T. spiralis* from doubly infected mice (*N. dubius* plus *T. spiralis*) to naive recipients was also impaired. In the majority of experiments reported here transfer of day 8 MLNC from doubly infected mice failed to transfer immunity whereas MLNC from singly infected donors (*T. spiralis* only) were usually successful in causing a significant reduction in mean worm recovery in recipients. Two experiments in which MLNC were taken from mice on day 20 of a double infection also failed to transfer immunity but cells from *T. spiralis* infected mice were also unsuccessful at this time.

The inhibitory effects of *N. dubius* also operated against the expression of immunity by competent cells. Transfer of cells from singly infected mice (*T. spiralis*) to mice harbouring a *N. dubius* infection did not result in an accelerated onset of elimination of *T. spiralis* from these animals. There was no evidence that these cells had any effect (in terms of expulsion) on *T. spiralis* even 12 days after transfer.

Two experiments designed to assess whether or not *N. dubius* caused suppression by favouring the production or action of suppressor cells provided equivocal results.

Removal of N. dubius from doubly infected mice had no effect on the expulsion of T. spiralis which still followed the delayed time course pattern. However when immune MLNC were given to doubly infected mice the cells did have some effect on T. spiralis when N. dubius had been removed suggesting that suppression requires the presence of the parasite.

The effect of N. dubius on the ability of mice to generate cells capable of transferring immunity was shown to involve both T and B cells. Transfer of day T-cell or B-cell enriched fractions of MLNC transferred immunity more effectively than did unseparated MLNC. However neither of these fractions were successful when the cell recipients harboured a pre-existing (8 week old) N. dubius infection.

The effects of N. dubius seemed to be against worm expulsion. Throughout these experiments N. dubius infections whether in donors or recipients, appeared not to interfere with the normal expression of anti-worm immunity (i.e. reduced fecundity and size) from transferred cells.

A number of experiments were carried out in attempts to determine the effect which N. dubius had upon the homing of i/v injected MLNC. The pattern of homing was similar in uninfected controls and in mice infected with N. dubius for 4, 8, 12 and 16 days. Enhanced localization of cells occured in the small intestine of mice infected with T. spiralis but only on day 4 of infection. Mice infected with both T. spiralis and N. dubius did not show this enhanced localization until day 12 of infection.

The ability of mice to mount an inflammatory response to a biologically inert stimulus i.e. a nitro-cellulose filter, implanted subcutaneously, was not affected by the presence of N. dubius. An

attempt to monitor macrophage activity by measuring peritoneal macrophage acid phosphatase activity was unsuccessful.

As a way of assessing whether or not cells in the Peyer's Patches, MLN and other organs were responding to infection mice were injected with a radiolabel which is incorporated only by dividing cells. Although there was increased incorporation in the MLN and spleen this was not evident when counts were considered on a weight for weight basis. N. dubius infection caused a depletion of the cell content of the MLN in the early part of the infection but cell numbers were restored and eventually rose above those of controls where they remained for the duration of the infection. This is in contrast to T. spiralis in NIH mice in which MLN blastogenesis peaks on day 4 of infection and the numbers of cells in the MLN rises rapidly between days 4 and 8 prior to the elimination of adult worms from the intestine.

Mitogen responsiveness of MLNC from T. spiralis, N. dubius and T. spiralis x N. dubius infected mice was depressed when compared to that of control MLNC. When cells from mice infected with T. spiralis and N. dubius were mixed control levels of responsiveness were restored.

Concentrated ES products from N. dubius infected mice did not have any lymphocytotoxic activity.

The evidence presented here and that from other studies (Chowaniec, Wescott and Congdon 1972, Shimp, Crandall and Crandall 1975, Jenkins and Behnke 1977, Behnke, Wakelin and Wilson 1978) demonstrate that N. dubius has a marked immunosuppressive effect on host responses to non-related antigens. This effect is known to be dose dependent (Jenkins and Behnke 1977, Behnke et al. 1978) and may also be time dependent, as in experiments with double infections of T. muris and N. dubius it is only when N. dubius is given within a few days of

T. muris is a delay in expulsion evident (Jenkins and Behnke 1977). Experiments suggest that it is the sensitization of cells against T. spiralis which is the process affected by N. dubius since MLNC taken from mice on day 8 of a double infection did not transfer immunity to naive recipients. However there is also evidence that the expression of primed cells is also prevented. Cells taken from immune donors i.e. mice infected with T. spiralis only do not transfer immunity to T. spiralis when the recipient mice harbour infections with N. dubius. Behnke et al (1978) have previously suggested that both afferent and efferent components of the immune response were affected by the presence of N. dubius and showed that memory of prior infection was abolished when the second T. spiralis infection was given concurrently with N. dubius.

Jenkins (1977) failed to find any evidence for the action of an N. dubius induced suppressor cells which delayed the expulsion of T. muris from mice. There is no reason to suppose that suppressor cells do not have important immunomodulant activity in helminth infections and indeed they have been implicated in reduced host responses against Ascaris suum in guinea-pigs (Khowry, Stromberg and Soulsby 1977), Brugia pahangi in jirds (Portaro, Britton and Ash 1976) and S. mansoni in rats (Camus, Dessaint, Fischer and Capron 1979). The results presented here are not so clear. There was evidence from one experiment that MLNC from N. dubius infected mice did interfere with MLNC from T. spiralis infected mice when the populations were mixed and given to naive recipients but a replicate experiment failed to give such convincing evidence. If the production or activity of a suppressor cell is enhanced by N. dubius infection the cell may be produced or active only during a restricted period of the infection.

The ideal time, as far as N. dubius is concerned, would be during the early tissue phase of infection when the parasite is most susceptible to immune attack. This might explain the suppressive action of the larval stage which is more potent than that of the adult and also explain why it is that the responses to other intestinal helminths are simply delayed rather than completely prevented. Of course the possibility that the expulsion that does take place in concurrent infections with other intestinal helminths is due to a completely different mechanism cannot be excluded.

Other possible mechanisms of parasite induced suppression which may be operative in the N. dubius-mouse system are considered below.

LYMPHOCYTOTOXIC FACTORS

Lymphocytotoxic factors have been found in the serum of mice infected with T. spiralis (Faubert and Tanner 1975). Goose (1975) demonstrated the in vitro toxicity of Fasciola hepatica ES-products for rat lymphoid cells and Komatsu, Nishimura, Sano and Shinka (1979) have suggested that the suppressive effects of a crude extract or maintenance fluid of Ascaris suum were due to lymphocytotoxic activity. Behnke et al (1978) suggested that N. dubius might also produce such a factor. Liu (1965) in his study of the pathology of N. dubius in C_3H and Webster mice reported that the damage caused to the liver, spleen and GALT could have been caused, in part, by a necrosin, a substance released by the growing larvae which lysed the surrounding tissue. The breakdown products of cell lysis might enhance the toxic effect on other cells and would have marked deleterious effects on immune responsiveness. However, the results presented in this study do not provide any evidence that N. dubius ES-products are toxic for lymphoid cells.

LYMPHOCYTE SUPPRESSIVE FACTORS

No evidence exists to show that suppressive factors are produced by N. dubius. Jenkins (1977) prepared a whole adult worm homogenate of N. dubius as a means of obtaining any suppressive factors which were present in the worms. However, multiple injections of the homogenate failed to suporess the immune expulsion of T. muris from mice. With Brugia pahangi in jirds Portaro, Britton and Ash (1976) were unable to isolate any suppressive factor from sera although the mitogen responsiveness of cells from these animals was lower than that of age matched controls. In contrast Weiss (1978) working with Dipetalonema viteae in hamsters demonstrated that serum from chronically infected animals depressed mitogen responsiveness of filarial-antigen-sensitive lymphocytes. However this serum factor was not characterized and may not have been of parasite origin. The problem with isolated materials such as these is that it is difficult to mimic the dose/rate of exposure found in the normal infection process and so the results should be interpreted with caution.

ANTIGEN INDUCED SUPPRESSION

There can be no doubt that antigen induced suppression occurs in N. dubius infected animals. It has already been mentioned that the parasite produces an array of antigenic material which must compete for space in the immune response. Day, Howard, Prowse, Chapman and Mitchell (1979) have shown by SDS-Page-analysis that N. dubius ES-products contained at least 17 proteins. The molecular weights of these proteins which ranged from 16.2 to 96.6 Daltons ($\times 10^{-3}$) make it possible that epitopes (antigenic determinants) on the same molecule (intra-molecular competition) as well as on different molecules (inter-molecular competition) might compete for space in the host response. The addition of another parasite only increases the possibility of

antigen induced suppression. Antigen induced suppression may occur at two levels (see Terry 1977). The first, involving non-specific suppressor cells has been mentioned earlier and is based on the hypothesis that suppressor cells are normally produced to limit a developing immune response. The non-specificity of their action, mediated at a distance by a lymphokine (see Waksman 1977) causes other cells to switch off DNA synthesis (Pross and Eidinger 1974) thus reducing their responsiveness to other antigens. The second level at which antigen induced suppression may occuris at the initiation of the immune response and the cell involved at this stage is thought to be the macrophage. Alteration of macrophage functioning during nematode infection has been reported on a number of occasions. Keller and Jones (1971) showed that peritoneal macrophages which had been activated by N. brasiliensis infection (or peptone injection) prevented the proliferation of Walker sarcoma cells, although co-operation with lymphocytes in the process was not excluded. IgG_2 from the antiserum of rats infected with N. brasiliensis prevented tumour cell engulfment by macrophages. It was suggested that the IgG_2 blocked antibody receptors on macrophages from responding to tumour-bound antibodies (Keller and Jones 1971). Macrophages from T. spiralis infected mice have been implicated in increased responses to particulate substances (Molinari, Cypess and Ebersole 1974). Increased phagocytosis by macrophages causes an increase in the rate of carbon clearance and enhances the resistance of infected mice to the intracellular bacterium Listeria monocytogenes (Cypess, Lubiniecki and Swidwa 1974). Resistance to Trypanosoma lewisi in rats (Meerovitch and Ackerman 1974) and to Babesia microti in mice (Carlaw, Phillips and Wakelin, in press) is also enhanced in T. spiralis infected animals. With the exception of the N. brasiliensis/tumour system mentioned above no other record of depressed macrophage responses during nematode

infection has been documented. A model of AIS has been proposed in which the macrophage plays an important part. It is hypothesized that macrophage functioning remains normal. The model describes T-lymphocyte interaction with antigenic determinants on foreign molecules and the release of a co-operating factor (antibody or antibody-antigen complex) which then attaches to Fc receptors on the macrophage which in turn present it to B-cells, triggering antibody production. If two antigens are administered sequentially then the factors released by T-cells (so called Ig-T) will be competing for limited macrophage Fc receptors and so the response to the second antigen may be depressed despite normal macrophage functioning (see Feldmann et al 1974).

The two examinations of macrophage activity carried out in this study were aimed at detecting differences in actual responsiveness of macrophages, theoretically the AIS model outlined above does not require depressed or enhanced macrophage activity to be effective, although changes in macrophage activity would obviously have an effect on the resulting response.

The acid-phosphatase test proved to be of little value. The high levels of activity in each macrophage (too high to be counted) may have been a characteristic of NIH mice, such high levels of macrophage enzyme activity if indicative of immune responsiveness may help explain the relative resistance of NIH mice to helminth infections. However, this conclusion would be valid only after extensive investigations in other strains of mice. The attempt to measure macrophage activity using the inflammation test was technically successful even if no differences were detected between control and N. dubius infected animals. If this test is to be used again mice with activated macrophages i.e. BCG or C. parvum stimulated should be included as a positive control.

The role of inflammatory responses in the expulsion of intestinal nematodes has been emphasized by Ogilvie and Jones (1971), Larsh and Race (1975) and Kennedy (1980). In the case of T. spiralis in mice any factor which altered or delayed the inflammatory response would almost certainly delay the expulsion of the parasite. The fact that the early polymorph response and the later macrophage response to filters in infected mice was not altered from that of control mice suggests that the effect of N. dubius is not against the cells involved in this type of inflammatory response. The possibility exists however, that as the filters measured a peripheral inflammatory response the effect of N. dubius at the intestinal level may have been missed. Arguing against a localized effect of N. dubius on the immune response is the work of Liu (1965), Jones (1974), Chowaniec, Wescott and Congdon (1972) and Jenkins and Behnke (1977) who demonstrated changes in the spleen, depressed responses to a virus and delayed rejection of the caecal dwelling nematode T. muris.

ALTERATION OF IMMUNOGLOBULIN PRODUCTION

Brown, Crandall and Crandall (1976) have suggested that the increased IgG_1 catabolism in mice infected with N. dubius is responsible for the depressed responses to heterologous antigens. Increased breakdown of IgG_1 apparently has an effect on the breakdown rates of other Immunoglobulin G subclasses. The production rates of immunoglobulins were not considered in the investigation. The biological and adaptive functions of IgG_1 are unclear but it is possible that when IgG_1 is overproduced the antibodies may swamp Fc receptors on macrophages preventing them from responding. Chapman, Knopf, Hicks and Mitchell (1979) and Chapman, Knopf, Anders and Mitchell (1979) demonstrated that the organs responsible for IgG_1 production were those 'in line' for antigen capture; the intestinal lymph nodes in

the case of N. dubius and the liver and spleen in the case of the larval cestode Mesocestoides corti. These workers were able to induce high numbers of IgG_1 anti-SRBC PFC in the spleens of mice in response to multiple high-dose injections of SRBC, i.e. a model of chronic antigen exposure. They concluded that the high IgG_1 levels found in N. dubius and M. corti infected mice reflected the chronic nature of the infections which gave a T-dependent stimulation of B cells.

DISTURBANCES OF LYMPHOCYTE TRAFFIC

A great deal of evidence has been gathered showing that lymphoblasts from the thoracic duct (Gowans and Knight 1964, Hall, Parry and Smith 1972), mesenteric lymoh nodes (Griscelli, Vassalli and McCluskey 1969, Guy-Grand, Griscelli and Vassalli 1974, Parrott and Ferguson 1974), and intestinal lymph (Hall, Hopkins and Orlans 1977) all migrate to the lamina propria of the small intestine. Alterations in the normal homing patterns of cells to the small intestine during nematode infections are also well documented (see Table 4). The exact function of the localization of lymphoblasts in the lamina propria is not known at present but Manson-Smith, Bruce and Parrott (1979) and Manson-Smith, Bruce, Rose and Parrott (1979) have shown that enhanced localization of mesenteric lymphoblasts in NIH mice during T. spiralis infection peaks between days 2 and 4 and precedes the initiation of villus atrophy and crypt hyperplasia. They have shown that villus atrophy and crypt hyperplasia is correlated with the expulsion of the parasite from the intestine. Their work has also shown that cell localization is predominantly of T-lymphoblasts and is highest in the region of the intestine occupied by the parasites.

The work reported here describes a major effect of N. dubius on the lymphoblast migration patterns in mice infected with T. spiralis. The only large changes between control and N. dubius infected mice,

were in the spleens of the latter which showed an increased isotope count, thus correlating well with the observed splenomegaly. The increased counts in the spleen were probably due to the increased number of macrophages as these are known to play a role in lymphocyte trapping (Frost and Lance 1974). There were no changes, from controls, in the degree of homing to the small intestine of mice infected with N. dubius alone. The enhanced migration to the small intestine observed in the doubly infected groups did not occur at day 4 as in mice infected with T. spiralis alone, but at day 12 (see Figure 25) and it is tempting to suggest that this change in the migration pattern is responsible for the delayed expulsion of T. spiralis. With reference to Figures 1 and 2 which show worm expulsion patterns and Figure 25 which shows isotope recoveries from the small intestine, it can be seen that in T. spiralis infected mice, and in doubly infected mice, the peak localization to the small intestine occurs 4 days prior to the onset of worm expulsion. As infection with N. dubius alone did not decrease the degree of cell homing to the small intestine it must be assumed that the migration of lymphocytes responding to T. spiralis infection was caused by an N. dubius-induced delay of the inflammatory changes normally associated with T. spiralis infection. Cells taken from the MLN of doubly infected mice at day 12 may have been capable of transferring immunity to T. spiralis whereas those taken earlier (see Figure 4) were not. Moore and Hall (1973) provided evidence that the nature of the inflammatory stimulus was important for localization of lymphoblasts and their results would support the suggestion that N. dubius has an effect on the inflammation in the gut. As well as having an effect on the inflammatory response induced by T. spiralis it is possible that the pathological changes in the spleen and lymph nodes in N. dubius infected mice (Liu 1965) may have altered the trapping properties of these organs but no evidence for this was found

other than that of increased spleen localization. Such effects have been suggested as possible causes of hyporesponsiveness in other systems where enhanced lymph node trapping of cells in response to antigen, suppressed responses to other antigens (Schlossman, Levin, Rocklin and David 1971). This was a transient effect, like the effect of N. dubius on the expulsion of T. spiralis. It is obvious that as the role of lymphoblast homing in gut immunity is still unclear, the precise relevance of the findings described here will have to await further investigation.

DEPRESSION OF LYMPHOCYTE RESPONSIVENESS

Although there is no evidence in the literature to show that N. dubius produces a lymphocyte suppressor factor, the study conducted here on lymphocyte responses to PHA did show that cells from infected mice did not respond as well as those from controls. In the final experiment on mitogen responsiveness in which cells were taken from mice infected with T. spiralis, N. dubius or T. spiralis plus N. dubius responses of MLNC from all these groups were lower than those of controls. A mixed cell population, cells from N. dubius and T. spiralis infected mice responded as well as control cells from uninfected mice. This might be explained in terms of the cell populations lacking a cell type necessary for stimulation. Only when the cells are mixed are there enough of the appropriate cells to give control stimulation values. As far as the depressed responses are concerned Ljungstrom (1980) has described similar depressed responses (of spleen cells) to polyclonal activators early in T. spiralis infection. The reduction in responsiveness was not due to a depletion of the T-cell dependent areas of nodes and spleen (Ljungstrom and Ruitenberg 1976) and in fact she favoured suppressor cells as being the most important contributory factor. The depressed mitogen responses in N. dubius infected mice

did not tie in with the *in vivo* isotope experiments where there was some evidence of blastogenesis in the MLN and spleen, nor with the cell counts in which there was an early decline followed by a steady rise to levels above those of controls. It was not possible to link these responses to the different phases of the *N. dubius* infection. This is in marked contrast to the study of *T. colubriformis* in guinea pigs (Dobson and Soulsby 1974) in which the lymphoblast response started in the Peyer's patches and moved rapidly into the lamina propria and MLN reaching a peak 6 days after infection a time when lymphocyte transformation to *T. colubriformis* antigens was first detected. All the lymphoblast responses were completed prior to the start of worm elimination.

It is possible that the reduction in lymphocyte responsiveness was due to the pre-committment of these cells to some of the many ES products of *N. dubius* (see Day *et al* 1979) and any of the mechanisms of AIS mentioned earlier may be responsible.

To conclude, the immunosuppression caused by infection with *N. dubius* which is active against a variety of heterologous antigens appears to be due to an effect of the parasite on lymphocyte responsiveness/effectiveness. The way in which this suppression is triggered is unclear.

Immunity to *N. dubius*

SECTION 2

INTRODUCTION

In most laboratory models of parasite immunity (see Table 1) a single exposure to the parasite is sufficient to protect the host against homologous challenge infection (Reviewed, Wakelin 1978, Mitchell 1979). Depending upon the parasite concerned protection can be expressed anamnestically in the form of accelerated worm expulsion (which may occur as early as 24 hours post challenge, Bell and McGregor 1979) or may take the form of a more rapid parasite attrition within the hosts tissues (Moqbel 1980). Many workers have demonstrated that this accelerated responsiveness could be transferred (with immune or sensitized MLNC and /or immune serum) to naive recipients (Keller and Keist 1972, Ogilvie and Jones 1968).

There have been relatively few attempts to analyse the cellular response to N. dubius, and the role of lymphoid cells has not received a great deal of attention. Various workers have reported on the involvement of peritoneal exudate cells, e.g. eosinophils and macrophages (in vitro and in vivo) in responses to the larval stages and in the cellular reaction that forms about developing larvae in immune mice (Jones and Rubin 1974, Jones 1974, Chaicumpa, Jenkin and Fischer 1977, Chaicumpa et al 1978, Prowse, Ey and Jenkin 1978). Only Prowse, Mitchell, Ey and Jenkin (1978) and Behnke and Parish (1981, in press) have examined the effect of transferring immune mesenteric lymph node cells on the survival of the parasite within the host. Before attempting to transfer immunity to naive recipients it is necessary to ensure that the immunising schedule used on donor animals has induced high levels of protection. One of the problems associated with N. dubius is the failure of single infections to induce the

high levels of protection against re-infection which are found in other laboratory model systems (see General Introduction); multiple infections are therefore required. Van Zandt(1962) used 3 repeated infections each of 50 parasites to immunise mice. He also immunised mice using preparations of larval or adult antigens but the protection induced was much less than that obtained following 3 oral infections. Behnke and Parish (1979a,1979b, 1981 in press) used a six dose infection schedule to stimulate an immunity which could be transferred synergistically with cells and serum. Prowse et al.(1978a) showed that, in their system, two immunising doses were required before mice became protected. Other workers have been able to stimulate immunity with larval stages injected intraperitoneally or subcutaneously (Cypess 1970a). This method requires very large numbers of parasites and may stimulate a different type of response from that initiated by multiple immunisation. One technique which has not been employed with N. dubius is the use of irradiated larval stages for immunisation.

The short term effects of radiation on biological systems have been extensively investigated mainly as a result of the use of ionizing radiation in medical diagnostic techniques. In fact early studies were aimed at destroying T. spiralis infective muscle larvae in pork (Tyzzer and Honeij 1916, Semrad 1937, Levin and Evans 1942). Stoner and Hale (1952) were first to report on the effect of irradiation on the ability of the host to mount an immune response against T. spiralis. Their pioneering work has led to the use of irradiation as a standard tool for the ablation of immune responsiveness to nematode parasites.

Jarrett, Jennings, McIntyre, Mulligan, Sharp and Urquhart (1959) were successful in immunising calves against Dictyocaulus viviparus

using an irradiated vaccine. Their classical study was the first to utilise an irradiation-induced curtailment of the parasite's life cycle at a specific, host-protective stage. Furthermore their work demonstrated that, at the correct dosage, the survival of larvae was such that while immunity was stimulated, much of the pathology associated with the disease could be prevented (Jarrett and Sharp 1963).

The success of 'Dictol' has not been repeated in other systems. Irradiated vaccines against Strongylus vulgaris, Ancylostoma caninum, Dictyocaulus filaria, Haemonchus contortus and Trichostrongylus colubriformis have all failed to become commercially viable (Ogbourne and Duncan 1977, Miller 1979, Movsesijan 1973, Mulligan, Gordon, Stewart and Wagland 1961) though for a variety of reasons.

More recently Taylor, James, Bickle, Doenhoff, Nelson, Hussein and Bushara (1977) have succeeded, in field trials, in preventing severe infections of S. matthei and S. bovis in sheep and cattle using an irradiated vaccine. Oothuman, Denham, McGreevy, Nelson and Rogers (1979) have also obtained partial immunization of cats against Brugia pahangi using a heavy immunising schedule of irradiated infective larvae.

The aim of the present work was to find a quick and effective method of stimulating immunity to N. dubius which would avoid the use of immunising schedules with normal larvae. The first part of this section deals with the use of multiple immunising schedules and the efficacy of cell and serum transfers from donors immunised in this way. An attempt at stimulating immunity non-specifically using the immunostimulant, Coumarin is also recorded as is the effect of immunisation with a field strain of N. dubius.

The final section assesses the effect of cobalt 60 irradiation on the growth and survival of *N. dubius* and the use of cobalt 60 irradiated larvae in the stimulation of immunity. Part of the work will be published in Parasite Immunology in a paper prepared in conjunction with Dr. J.M. Behnke and Miss Heather A. Parish. This work is consolidated with further studies on the use of irradiated larvae to stimulate immunity in various strains of mice.

SECTION 2

MATERIALS AND METHODS

EXSHEATHMENT OF LARVAE

Larval N. dubius can be exsheathed by dilute solutions of Sodium hypochlorite (Rubin, Leuker and Andersen 1968). After several trials, in which larvae were incubated in NaOCl at various concentrations and temperatures, it was found that at 37 C a 0.1% w/v Sodium hypochlorite, 0.875% w/v Sodium chloride solution (i.e. 5% Milton 2 sterilising fluid, Richardson-Merrell Ltd.) was 95% effective within 5 minutes. Exsheathing was carried out by placing a larval suspension (usually 1000 larvae/ml) in a 50ml beaker and adding Milton 2 (Sodium hypochlorite/Sodium chloride) solution to give the required final concentration. Larvae were washed in distilled water after exsheathment and resuspended in distilled water prior to injection.

IRRADIATION OF LARVAE

Unless otherwise stated the irradiation of larvae was carried out in the Department of Veterinary Physiology, University of Glasgow Veterinary School using a Cobalt 60 source. A stock larval suspension was adjusted to give the required infective dose in 0.2ml of suspension, aliquots, usually 10ml, were placed in plastic universal containers and these were lowered into the source for the required length of time (1.0K.rad/minute). After irradiation the contents of the tubes were transferred to conical flasks and a magnetic stirrer was used to disperse the larvae prior to inoculation.

CELL AND SERUM TRANSFERS

These were carried out as described in General Materials and Methods and Section 1 Materials and Methods.

FIELD STRAIN PARASITE

As described in General Materials and Methods.

COUMARIN

Coumarin (1-2 benzo-pyrone, Sigma) was administered i/p in 5% A.R. ethanol in physiological saline at a dose of 1mg/mouse on a daily basis. Controls received 5% A.R. ethanol in physiological saline. Coumarin is a potent stimulator of both macrophage activity and macrophage numbers (summarised Piller 1978).

SURGICAL TECHNIQUES

a) LAPAROTOMY

In some experiments animals were infected with adult parasites which were inserted directly into the duodenum.

Mice were anaesthetised with a mixture of 'Sagatal' (Sodium pentobarbitone 60mg/ml, May and Baker Ltd., Dagenham), 95% ethanol and HBSS in the ratio of 1:1·5:9 by volume. This solution was administered i/p, 0.01ml per gram of body weight. The anaesthesia persists for 2-3 hours. Operations were carried out within 1 hour of administration of the anaesthetic.

Once the anaesthetic had taken effect (1-2 minutes) the mouse was shaved and fixed to the operating surface using plastic tape and the skin of the abdomen sterilised with 70% ethanol. A small incision, approximately 1cm long was made with a scalpel to the right of the animal's midline 1cm below the edge of the ribcage. The body wall was pierced with a scalpel and the opening was extended using sharp forceps. The proximal duodenum was pulled through the opening using a small metal hook. The duodenum was punctured with a hypodermic needle and the worms were introduced by insertion of a drawn out glass pipette (O.D. 2mm). The worms were expelled into the

intestine, away from the stomach in 0.2ml HBSS. After removal of the pipette the puncture was sutured with a single stitch (0.7M Mersilk Mersuture, Ethicon Ltd., Edinburgh). The peritoneal cavity and exposed intestine were sprayed with an antibiotic mixture of bacitracin, neomycin and polymixin (Rikospray, Riker Laboratories Ltd., Loughborough). The intestine was replaced in the body cavity and the body wall closed with another single stitch and sprayed once again with Rikospray. The skin was closed with 2-3 sutures, sprayed with Rikospray and sealed with aerosol plastic skin (Nobecutane, Astra Chemicals Ltd., Watford). Blood loss was negligible and mortalities (usually due to anaesthesia) were rare. Antibiotics were provided in the drinking water of operated animals (see General Materials and Methods).

b) SPLENECTOMY

Following anaesthesia mice were placed on the operating surface in the right lateral position (left side uppermost). Both right limbs were taped perpendicular to the body axis. The left foreleg was taped after stretching it up (45°) towards the head. The left hindleg was stretched down towards the tail before taping. The abdomen was swabbed with 70% alcohol and then shaved as previously described. An incision was made in the skin just below the left costal margin and this was followed by an incision in the body wall (0.5-1.0cm) to expose the spleen. The spleen was exteriorized with brain forceps. Following exteriorization a pair of fine forceps was inserted through the mesenteric attachment on the underside of the spleen. Care was taken to avoid piercing the blood vessels of the upper and lower poles. Once through the mesentery a looped silk thread (sterile) was pulled back through and cut into two. One piece was pulled down to the lower vascular bundle and the second to the upper

vascular bundle. These were tied off. The spleen was cut away and the ligatures were trimmed.

Sham operated animals were treated identically up to the stage of exteriorization. The spleen was then re-inserted and the body wall and skin closed as described for laparotomy.

Mice were usually given fourteen days to recover from the operation before infection.

SECTION 2

RESULTS

Before attempting cell and serum transfers from N. dubius infected mice the time course of a primary and secondary infection was established.

In the first experiment, 44 6-week old female NIH mice were infected with 400 N. dubius on day 0 and groups of six or seven of these mice were killed on days 10, 14, 16, 18, 20, 30 and 40. The adult worm recoveries (Experiment 30) are shown in Figure 2-1.

The results show that there was no evidence of worm loss from these mice in the first 40 days of infection. Experience has shown that most worms migrate into the gut lumen from the mucosa on days 8-10 of infection (see General Introduction). On this occasion only about 50% of the inoculum was recovered. This rose to a plateau of about 60% over the next ten days.

There was no significant difference between the numbers of male and female recovered. Throughout the experiment the sex ratio was maintained at approximately 1:1.

CHALLENGE INFECTION OF N. DUBIUS IN MICE PREVIOUSLY IMMUNISED BY INFECTION.

This experiment was designed to establish the effect of an immunising primary infection on a subsequent challenge infection. Also included were groups of mice given pyrantel embonate (PYR) to test the efficacy of the drug for future use. Forty 8-week old NIH mice were used. The experimental design (Experiment 31) is set out below:

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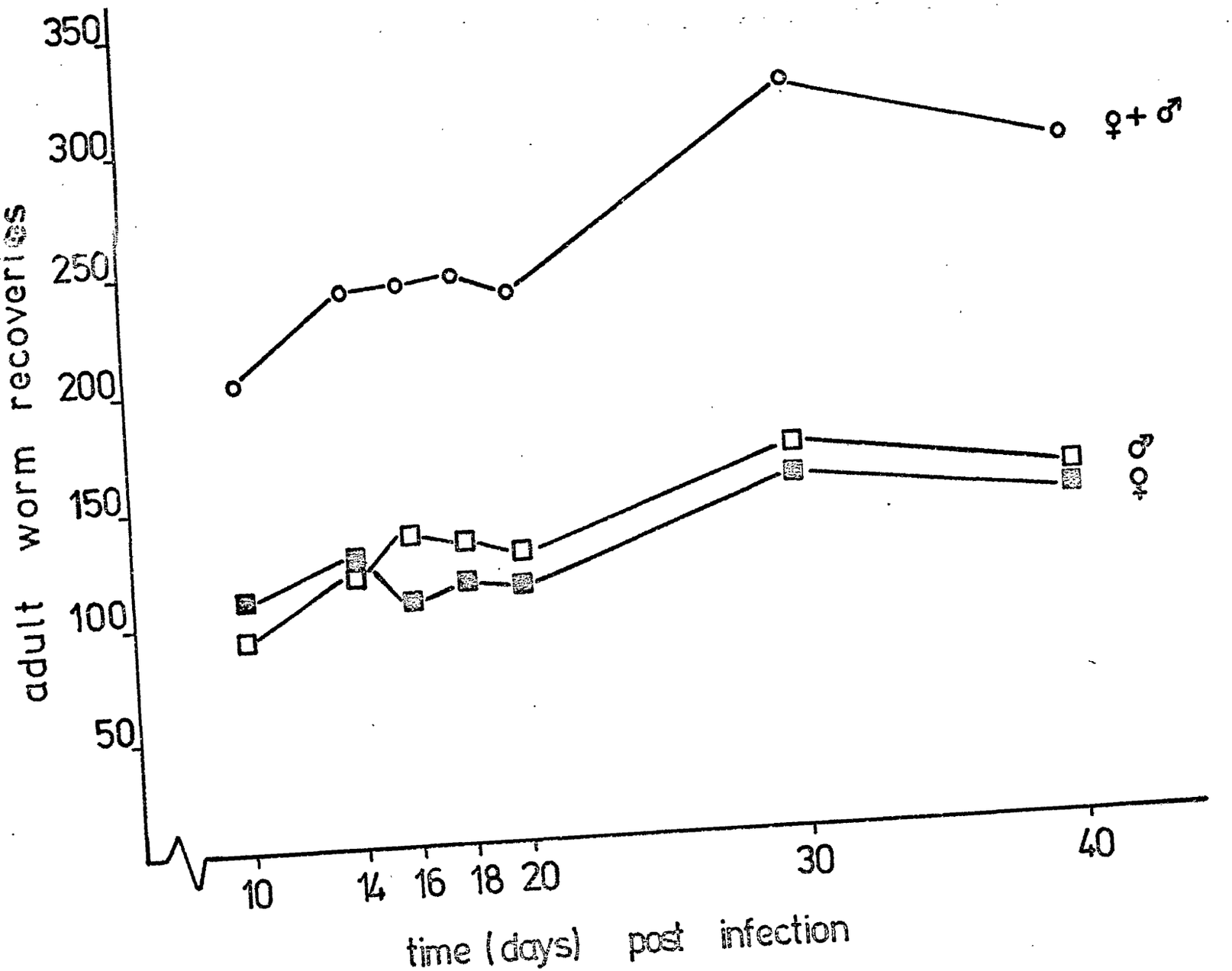
FIGURE 2-1

Primary infection of N. dubius in female NIH mice

Adult worm recoveries (Mean) at 10, 14, 16, 18, 20, 30 and 40 days post infection with 400 N. dubius

- O Total number of worms recovered (♀ and ♂)
- □ Male worms recovered
- ■ Female worms recovered


350
300
250
200
150
100
50
adult worm recoveries
♀+♂
♂♀
10
14
16
18
20
30
40
time (days) post infection

GROUP	DAY				
	0	14	18	19	35
A	Nd	PYR	PYR	Nd	K
B	Nd	PYR	PYR	K	K
C	Nd	PYR		K	K
D	Nd	-	-	K	K
E	-	PYR	PYR	Nd	K
F	-	-	-	Nd	K

Mice were infected with 400 N. dubius on each occasion and groups were killed on day 19 of primary infection and on day 16 of challenge infection (35 of primary). The worm recoveries are shown in Figure 2-2.

As far as the use of pyrantel is concerned, two doses on days 14 and 18 of infection (Group B) proved to be 100% effective as assessed by kills on days 19 and 35. The single day 14 dose (Group C) was less effective in that some worms were recovered on day 19 but all these worms were from a single mouse. Pretreatment with pyrantel (Group E) had no effect on subsequent establishment of N. dubius the mean worm recover being identical to those of Group F, the challenge control. Mean worm recovery from the primary infection control (Group D) was similar at both 19 and 35 days post infection. Group A, the group immunised with 400 N. dubius drug treated and subsequently re-infected had significantly fewer worms than the challenge control (Group F). This reduction in worm recovery, approximately 41% must be attributed to the effects of the prior immunisation. It is unlikely however, that the reduction was caused by loss of adult worms from the gut. Immunity to N. dubius can be expressed in a number of ways and the possibility that worm development and emergence had been/or can be delayed must be considered likely and has

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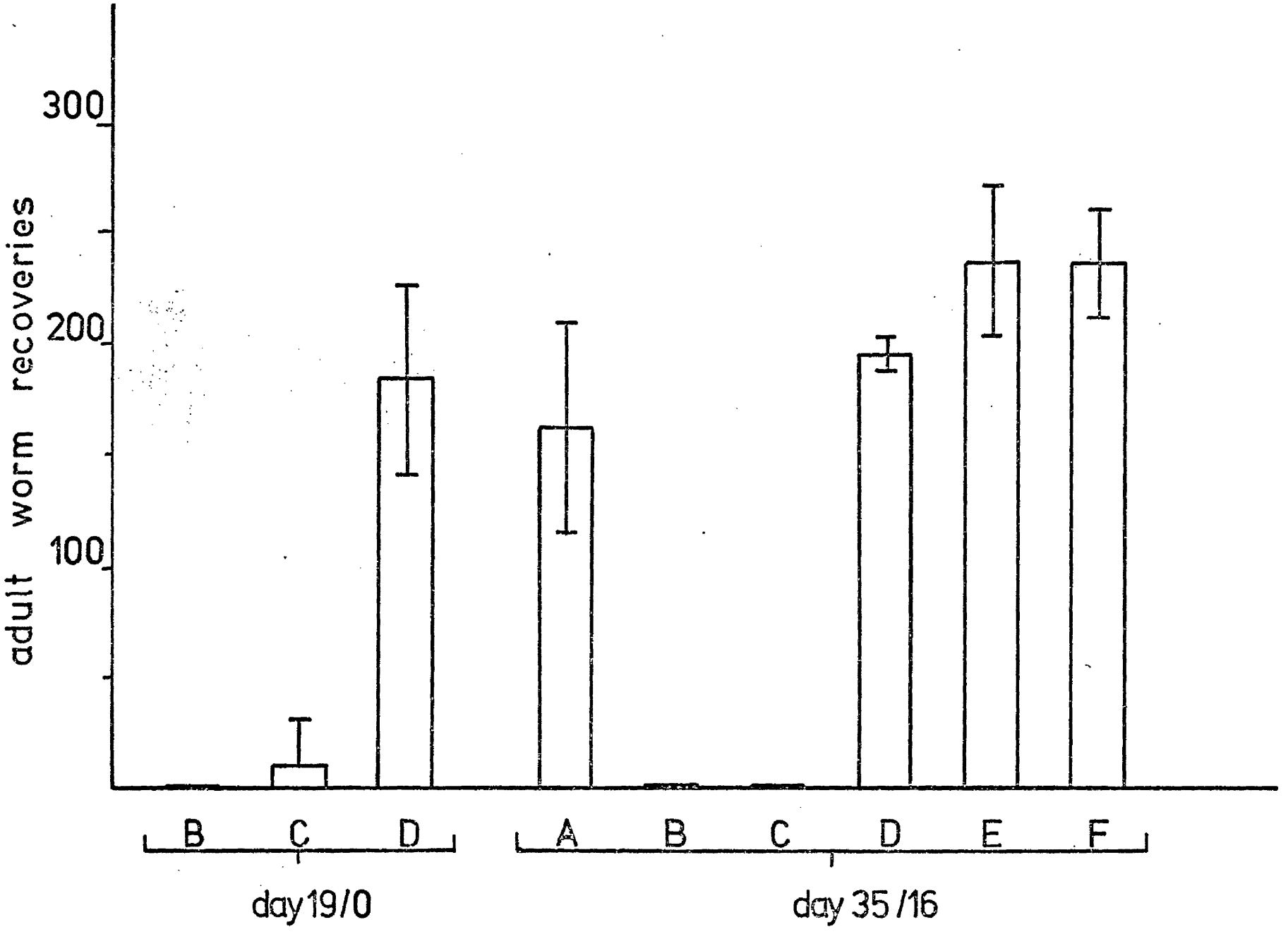
FIGURE 2-2

Challenge infection of N. dubius in mice previously immunized by infection

Mean recovery (MWR ± SD) N. dubius

GROUP	DAY				
	0	14	18	19	35
A	Nd	PYR	PYR	Nd	K
B	Nd	PYR	PYR	K	K
C	Nd	PYR		K	K
D	Nd	-	-	K	K
E	-	PYR	PYR	Nd	K
F	-	-	-	Nd	K


adult worm recoveries
300
200
100
B
C
D
A
B
C
D
E
F
day 19/0
day 35/16

an important bearing on this result. Having only one post-challenge kill makes it impossible to determine if this was in fact what had happened.

STIMULATION OF IMMUNITY WITH DIVIDED INFECTIONS

As was mentioned earlier (see General Introduction) multiple immunization schedules are usually required to stimulate protective immune responses to N. dubius. The success of multiple immunizing schedules can be attributed to the increased exposure of the host to larval antigens with each succeeding infection. To test whether or not increased exposure to the larval stages did produce better protection an experiment (Experiment 32) was carried out in which male CFLP mice were immunized with either 3 infections of 200 N. dubius or 6 infections of 100 N. dubius. The infections were given at intervals as shown below and each was terminated by pyrantel treatment to prevent the accumulation of lethal worm burdens in the intestine. Serum taken from these animals after the multiple immunizations was used in subsequent serum transfer experiments to assess the role of serum factors in immunity to N. dubius. The multiple immunising schedules were uncontrolled in that worm recoveries were not determined and no control infections given. However based on egg counts taken at two points in the immunising schedule, some measure of immunity did develop. Group 1 mice were given infections of 200 N. dubius on days 0, 19 and 38 treated with pyrantel on days 15, 34 and 53. Mice were killed and serum collected on day 54. Group 2 mice were given infections of 100 N. dubius on days 0, 4, 8, 21, 24 and 28 and treated with pyrantel on days 17, 20, 37 and 40. Mice were killed and serum collected on day 41. Egg counts on days 14 and 52 for Group 1 and days 16 and 36 for Group 2 are shown in Table 6. There was a large reduction in egg counts from the mice immunised with

TABLE 6

Egg counts (e.p.g. x 10^{-3}) from mice immunized with 3 x 200 normal *N. dubius* larvae (Group 1) and 6 x 100 normal *N. dubius* larvae (Group 2)

DAY	14	16	36	52
GROUP 1	40	-	-	22
GROUP 2	-	53	9	-

6 x 100 N. dubius but although they were given the same total number of parasites the group given 3 x 200 N. dubius did not show a comparable reduction.

SERUM TRANSFER

The serum collected from the mice given multiple immunising infections (above) was used in two experiments. The experimental design for both experiments is shown below:

GROUP (N=6) DAY

	-1	0	+1	+6	+8	+12	+14	+20
A	I	100 Nd	I					K
B	C	100 Nd	C					K
C		100 Nd		I	I			K
D		100 Nd		C	C			K
E		100 Nd				I	I	K
F		100 Nd				C	C	K
G		100 Nd						K

Seven week old female NIH mice were given 1ml of 'immune' I, or control C, serum (collected from uninfected CFLP mice) i/p on the days specified. The times were chosen to assess the effect of serum on establishing larval stages, (days -1, +1), on pre-emergence juvenile parasites (days +6, +8) and on the lumen-dwelling adult parasites (days +12, +14). The results are shown in Figure 2-3, serum pool 1, (Experiment 34) and in Figure 2-4, serum pool 2 (Experiment 34a). Worm recoveries from the treated groups are expressed as percentages of control recoveries.

Both pools of serum were effective against N. dubius in terms of numbers of worms recovered, and the greatest effect of both was shown in the serum administered on days -1 and +1 or +6 and +8, i.e. when establishing larval stages and the pre-emergence juvenile

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FIGURE 2-3

Effect of serum transfer on N. dubius infection

(Serum pool 1 donors immunized 6 x 100 N. dubius)

Worm recovery as percentage of control Group G

(not illustrated)

GROUP	DAY and TYPE OF SERUM ADMINISTERED	
A	-1, +1,	immune (I)
B	-1, +1,	control (C)
C	+6, +8,	immune
D	+6, +8,	control
E	+12, +14,	immune
F	+12, +14,	control
G	no serum	

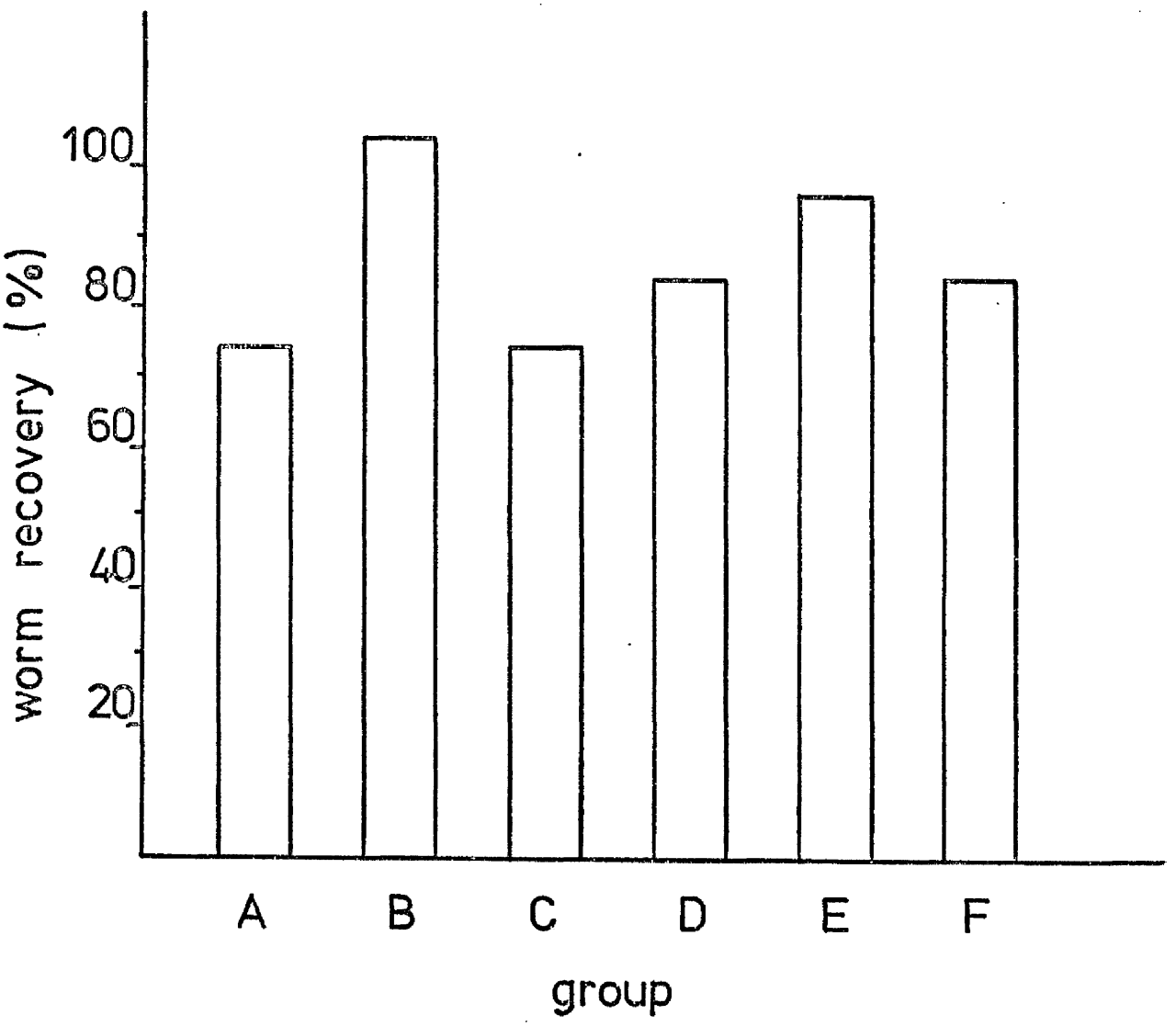
worm recovery (%)
100
80
60
40
20
A
B
C
D
E
F
group

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FIGURE 2-4

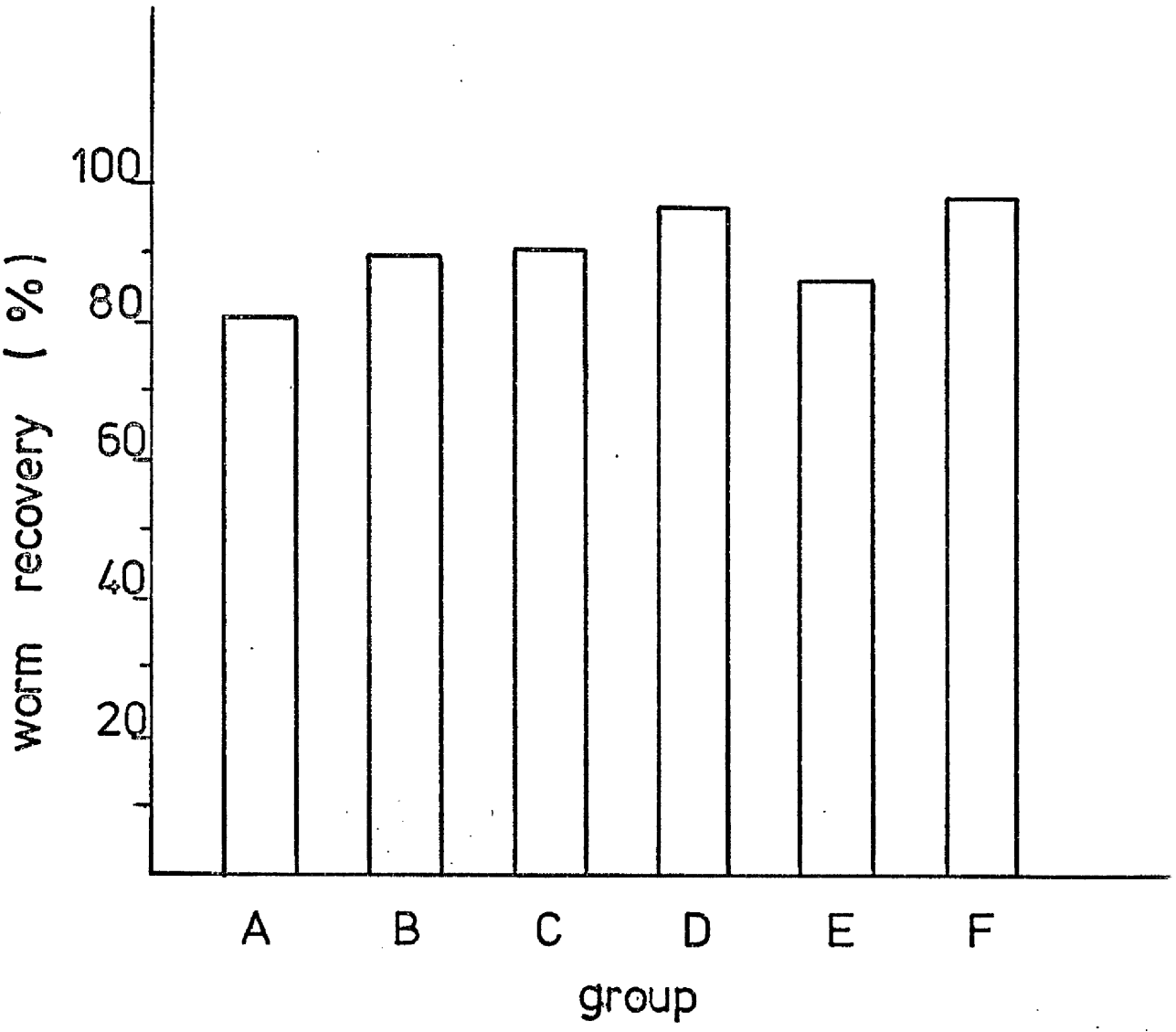
Effect of serum on *N. dubius* infection

(Serum pool 2 donors immunized 3 x 200 *N. dubius*)

Worm recovery as percentage of control Group G

(not illustrated)

GROUP	DAY and TYPE OF SERUM ADMINISTERED
A	-1, +1, immune (I)
B	-1, +1, control (C)
C	+6, +8, immune
D	+6, +8, control
E	+12, +14, immune
F	+12, +14, control
G	no serum


worm recovery (%)
100
80
60
40
20
A
B
C
D
E
F
group

parasites were present. The serum administered post adult emergence was ineffective. The effects however, were small, only a 26% reduction at best.

Reports from other laboratories have shown that serum pools taken from animals immunised in the same way have variable ability to protect naive recipients. To assess whether or not the serum did contain any anti-worm antibodies a double diffusion in agar gel was run between the serum pools and a saline extract of whole adult worm (homogenised). In both cases this showed at least one cross reaction. However such a test is not a good measure of '*in vivo*' activity. In view of the variability mentioned previously and since the mice in this experiment had been given such a heavy immunising infection schedule with such a poor effect, it was decided to discontinue serum transfers.

CELL TRANSFER

Having had only limited success with serum transfers a series of cell transfers was undertaken. In the first of these a multiple immunisation schedule was employed to prime donors prior to the collection of MLNC and SC and then transfer to recipients. Twelve 6 week old NIH mice were infected with 100 *N. dubius* on days 0, 3, 5 and 7 followed by pyrantel treatment on days 14 and 15. Two days later the mice were re-infected with 300 *N. dubius* and after seven days they were killed and their MLN and spleens removed for transfer. Recipients (12 per group) received 1.5×10^7 MLNC or 1.5×10^7 SC and, together with a group of control animals, were infected with 100 *N. dubius* on the same day. Mice were killed in groups of 6, except for the MLNC group in which only 2 were killed on day 14. This was because there had been a number of casualties immediately on injection of MLNC. The results, mean adult worm recoveries, on

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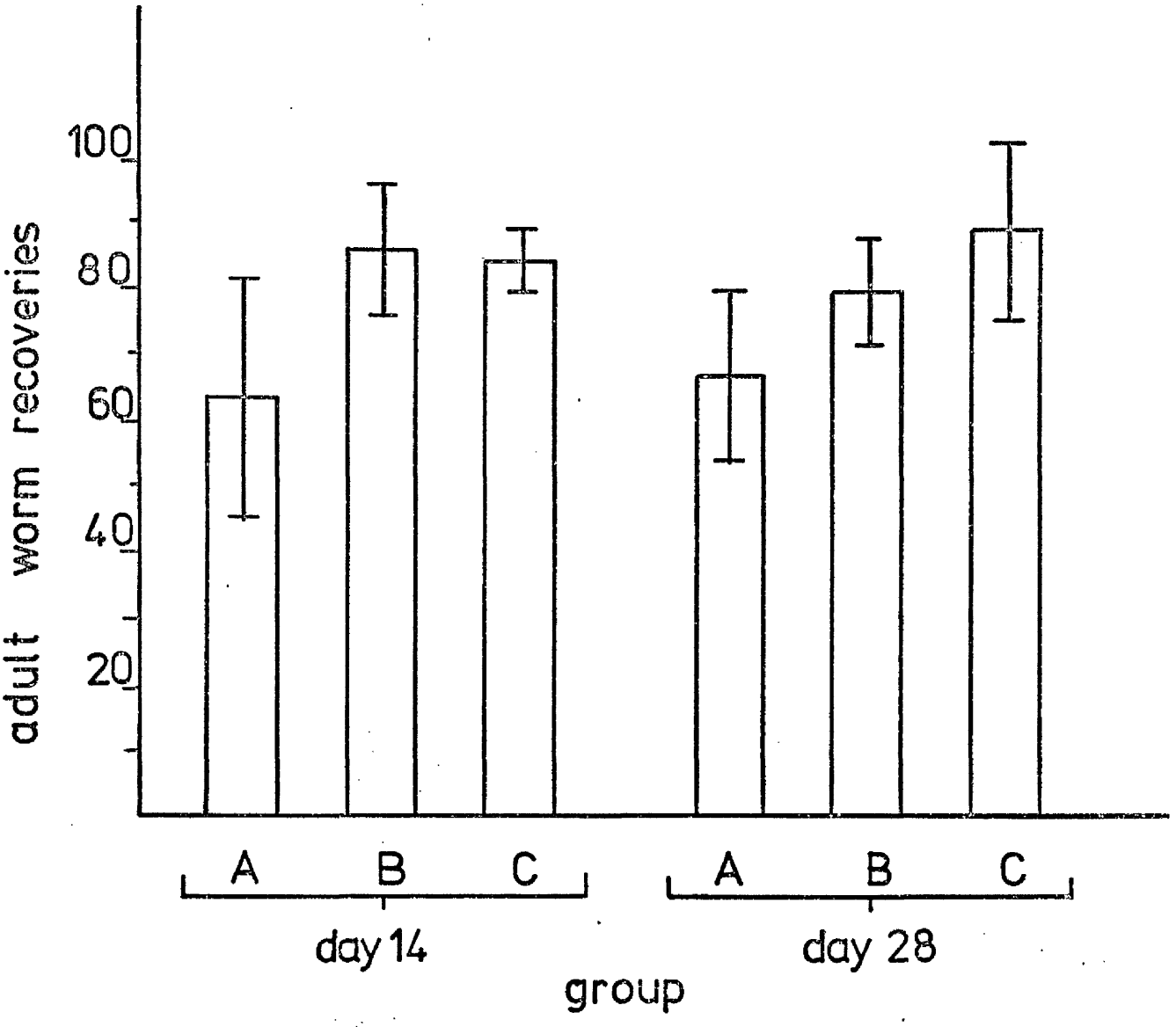
FIGURE 2-5

Cell transfer

Worm recoveries (MWR $\pm$ SD) *N. dubius* days 14 and 28 post infection with 100 *N. dubius*

A	1.5×10^7 MLNC	+ 100 *N. dubius*
B	1.5×10^7 SC	+ 100 *N. dubius*
C	Control, no cells	+ 100 *N. dubius*

Cell donors given 100 *N. dubius* on days 0, 3, 5 and 7, drug treated days 14 and 15. Re-infected 300 *N. dubius* day 17, killed and cells used for transfer on day 24.


adult worm recoveries
100
80
60
40
20
A
B
C
A
B
C
day 14
day 28
group

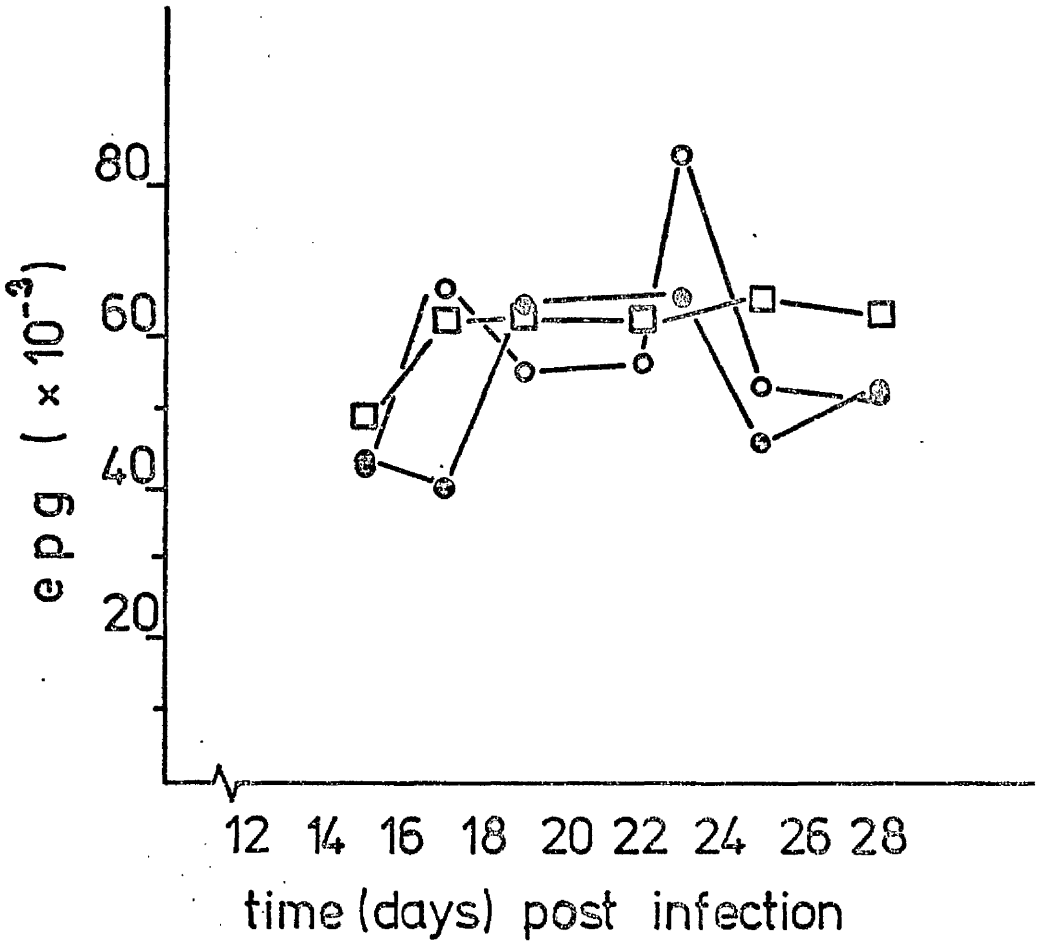
Facing page 146

FIGURE 2-6

Egg counts (e.p.g. x 10^{-3}) from groups on Figure 2-5

GROUP

A	●	1.5×10^7 MLNC	+ 100 N. dubius
B	□	1.5×10^7 SC	+ 100 N. dubius
C	○	Control, no cells	+ 100 N. dubius


epg (x 10^{-3})
80
60
40
20
12 14 16 18 20 22 24 26 28
time (days) post infection

days 14 and 28 post transfer/infection and egg counts (e.p.g.), are shown in Figures 2-5 and 2-6 respectively (Experiment 35).

The mean adult worm recovery in Group A, (MLNC transfer) was lower than that of controls on days 14 and 28 but once again the reduction was only of the order of 24.9% on day 14 and 26% on day 28. The SC transfer group (Group B) had a mean worm recovery similar to that of control mice on day 14 but was slightly lower, by 10.6% on day 28. As with serum transfer, the cell transfer was only marginally effective. The egg counts for these three groups were similar throughout the whole of the infection although those from the MLNC transfer group tended to be lower, but not significantly, than those of the control group.

In the second cell transfer experiment (Experiment 36) cell donors (NIH) were immunised by injection (i/p) of 1 x 4000 exsheathed larvae. Donors were killed 23 days after inoculation and MLNC and SC were prepared for transfer. Groups of male NIH mice were injected (i/v) with 1.5×10^7 MLNC or 1.5×10^7 SC and together with controls were infected with 100 N. dubius on day 0. Recipients were killed on days 14 and 28 and the results, mean adult worm recoveries and egg counts are shown in Figures 2-7 and 2-8 respectively.

The mean adult worm recoveries followed a pattern similar to that of the previous experiment (Experiment 35). In the MLNC transfer group they were reduced by 20% on days 14 and 28. The SC transfer group showed only a 5% reduction in mean worm recovery compared with control at day 28 only. The egg counts were consistently lower than in the previous experiment and again those from the MLNC transfer group tended to be lower than controls throughout the experiment. This result was not as good, in terms of protection, as some of those recorded by other authors using a similar approach.

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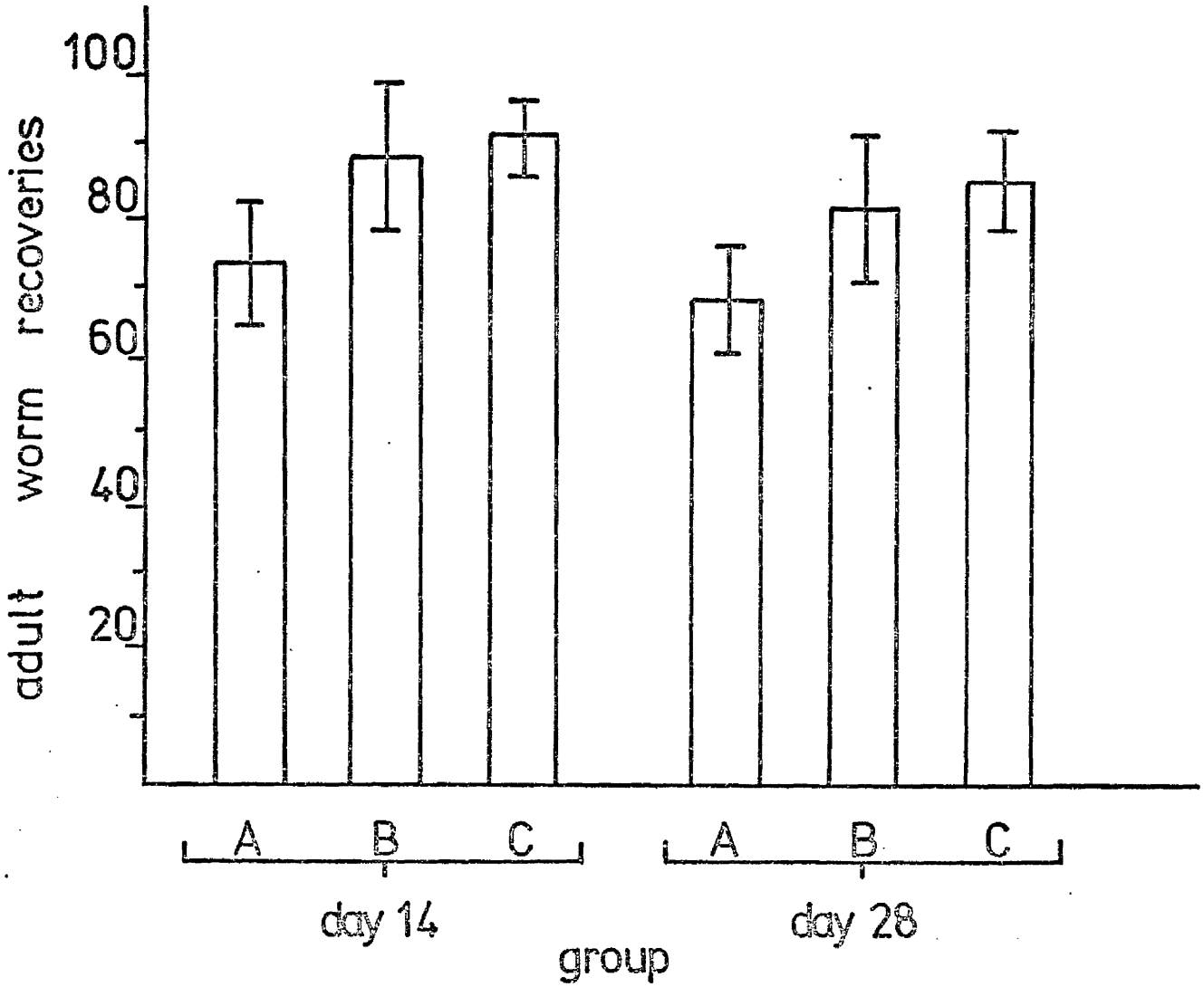
FIGURE 2-7

Cell transfer

Worm recoveries (MWR $\pm$ SD) *N. dubius* days 14 and 28 post infection with 100 *N. dubius*

A	1.5×10^7 MLNC	+ 100 *N. dubius*
B	1.5×10^7 SC	+ 100 *N. dubius*
C	Control, no cells	+ 100 *N. dubius*

Cell donors given 1 x 4000 exsheathed larvae *N. dubius* i/p
Killed and cells used for transfer on day 23.


adult worm recoveries
100
80
60
40
20
A
B
C
A
B
C
day 14
day 28
group

Facing page 149

FIGURE 2-8

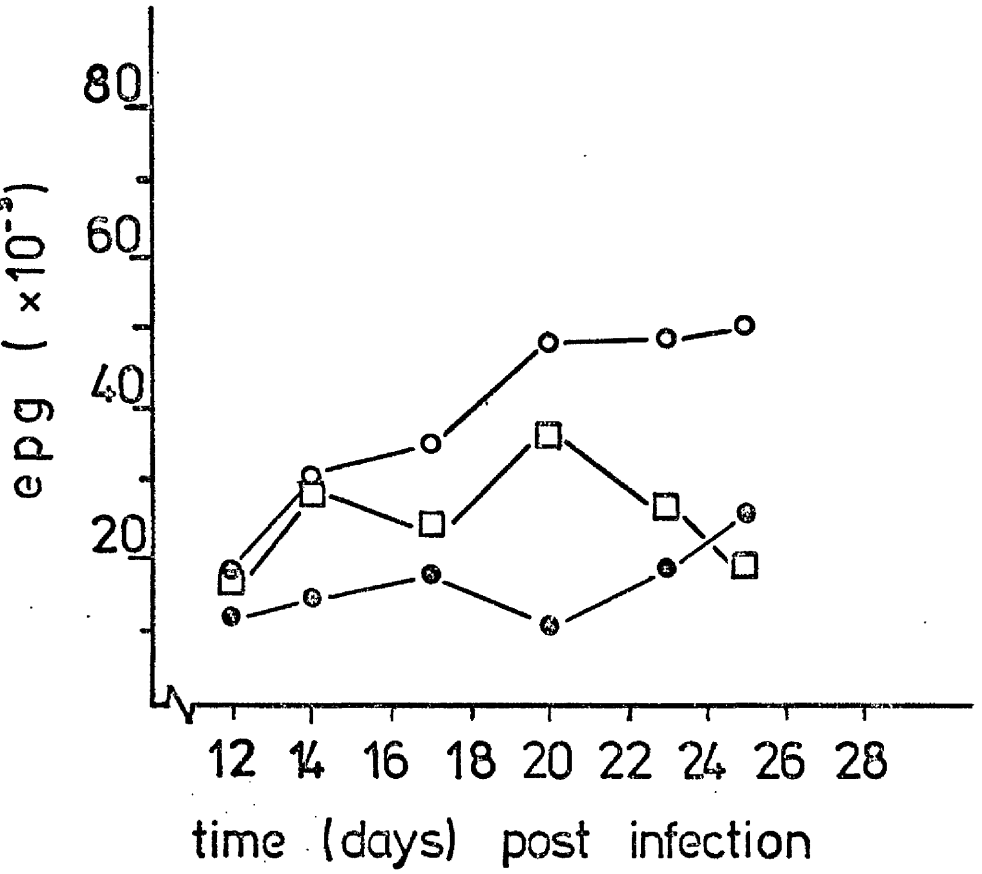
Egg counts (e.p.g. x 10^{-3}) from groups in Figure 2-7

GROUP

A ● 1.5×10^7 MLNC + 100 N. dubius

B □ 1.5×10^7 SC + 100 N. dubius

C ○ Control, no cells + 100 N. dubius


epg (×10⁻³)
80
60
40
20
12 14 16 18 20 22 24 26 28
time (days) post infection

The results from the cell and serum transfers were not sufficiently clear-cut to allow a more detailed analysis of the components involved in the immune response against N. dubius. As an alternative to the immunising schedules which were employed, an immunostimulant drug was administered to mice during N. dubius infection in an attempt to boost the response against the parasite to levels which would allow effective cell transfer experiments. The drug used was Coumarin (5,6 Benzopyrone) which has an enhancing effect on the mononuclear-phagocyte system causing numbers and activity of macrophages to increase. Coumarin was given at a dose of 1 mg/mouse in 5% ethanol in physiological saline on each day of infection. Controls received no treatment. Both groups of six, six week old male NIH mice were infected with 100 N. dubius and killed on day 20. The numbers of worms recovered (means) and egg counts (e.p.g.) throughout the course of the experiment (Experiment 37) are shown in Figures 2-9 and 2-10 respectively. There was no difference in egg count between groups during the experiment. Adult worm recoveries were high for a 100 worm infection and there was no significant difference between the groups at autopsy, possibly because of the variation in the worm counts which may have obscured any difference which existed. The mean worm recovery for the Coumarin treated group (Group B) was 20% lower than that of the controls.

As the previous experiment had not included a group given 2% ethanol in physiological saline the experiment was repeated. Eighteen male NIH mice, in groups of six, were infected with 100 N. dubius on day 0. Group A received daily injections (i/p) of Coumarin in alcohol from day -2. Group B received daily injections of alcohol and Group C received no injections. Mean adult worm recoveries

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FIGURE 2-9

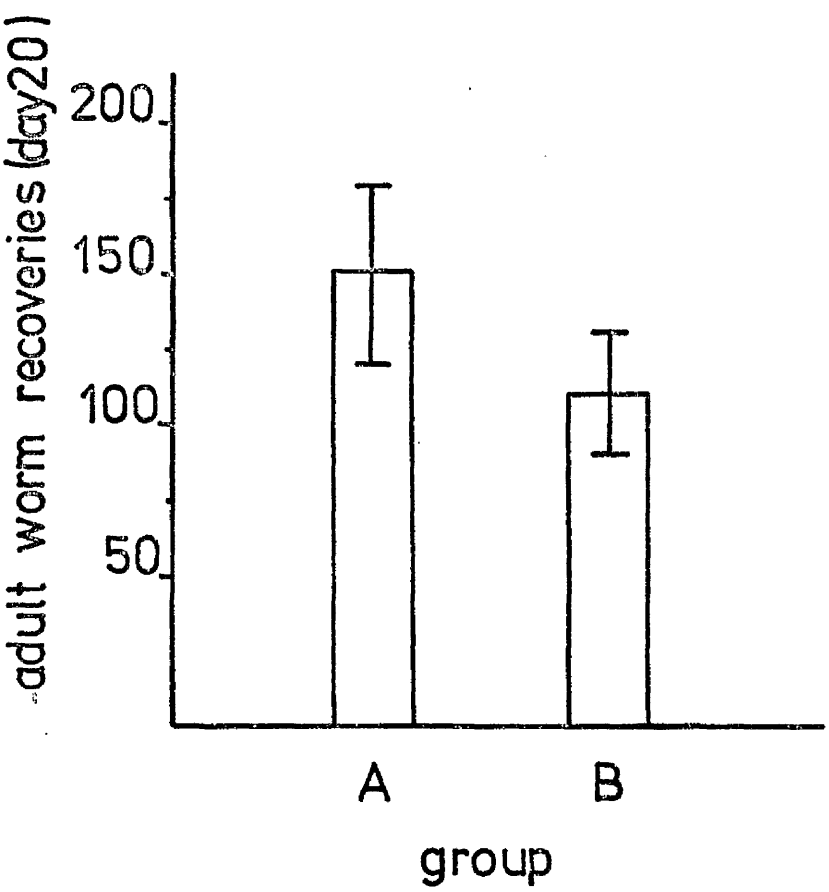
Effect of Coumarin (1,2 Benzopyrone) on N. dubius infection

Worm recoveries (M/R $\pm$ SD) N. dubius day 20 post infection with 100 N. dubius

GROUP

A 100 N. dubius

B 100 N. dubius + Coumarin (1 mg/mouse/i.p in 5% alcohol in physiological saline daily).


adult worm recoveries (day20)
200
150
100
50
A
B
group

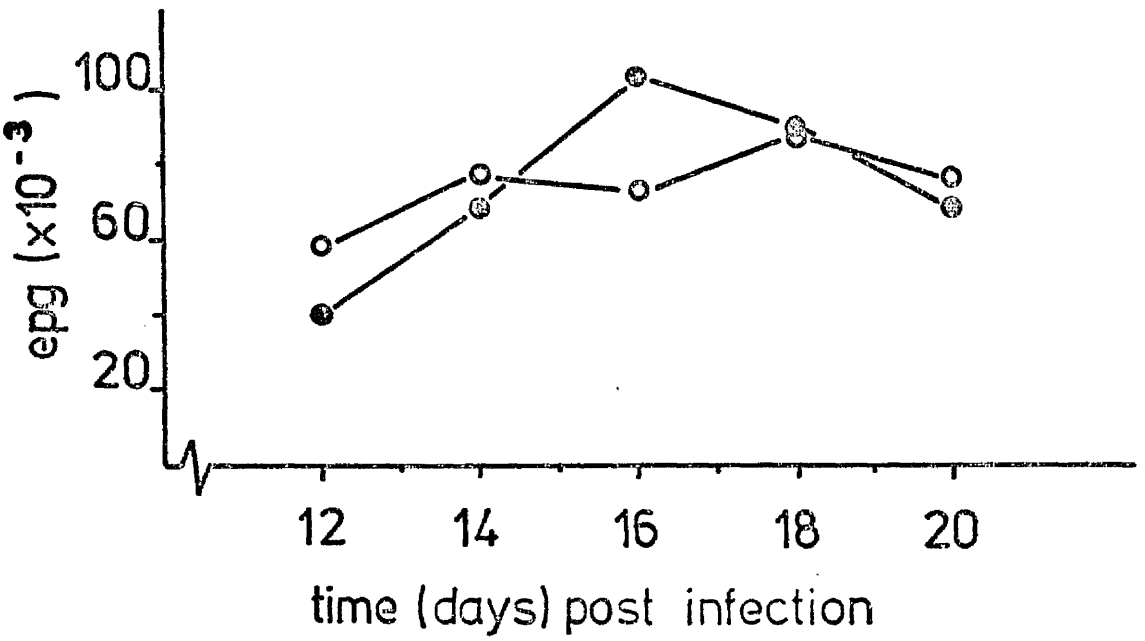
Facing page 152

FIGURE 2-10

Egg counts (e.p.g. x 10^{-3}) from groups in Figure 2-9

GROUP

- O 100 *N. dubius*
- ● 100 *N. dubius* + Coumarin


epg (x10⁻³)
100
60
20
12
14
16
18
20
time (days) post infection

(day 20) and egg counts (e.p.g.) are shown in Figures 2-11 and 2-12 (Experiment 38) respectively. The egg counts from all these groups were similar throughout the experiment. The day 20 mean adult worm recoveries did not differ statistically.

EFFECT OF SPLENECTOMY ON N. DUBIUS INFECTION

As a final attempt to stimulate an effective response against N. dubius the course of a primary and secondary infection of N. dubius was examined in splenectomised mice. Splenectomy is known to have a marked effect on the responsiveness of the host. In this case it was used in an attempt to boost the mononuclear phagocyte system. Splenectomy is known to increase phagocytosis at other sites in the animal and in addition to remove any potential suppressive factors or cells originating in or resident in the spleen.

PRIMARY INFECTION

Twelve seven week old male NIH mice were infected with 300 N. dubius on day 0. Two weeks earlier six of these mice had been splenectomised (see Materials and Methods). The egg counts from both groups were followed from day 12 to day 20. Mice in both groups were killed on day 20 and adult worms recovered. The results, mean adult worm recoveries, and egg counts are shown in Figures 2-13 and 2-14 respectively (Experiment 39).

There was no significant difference in mean adult worm recoveries on day 20. However, the egg counts, which had been similar throughout the experiment changed dramatically on day 20. There was a sudden 50% drop in the egg count from the splenectomised group. The final egg count was made post-mortem so it was impossible to say whether this had been a technical error or a real effect on the adult female worms.

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FIGURE 2-11

Effect of Coumarin (1,2 Benzopyrone) on *N. dubius* infection

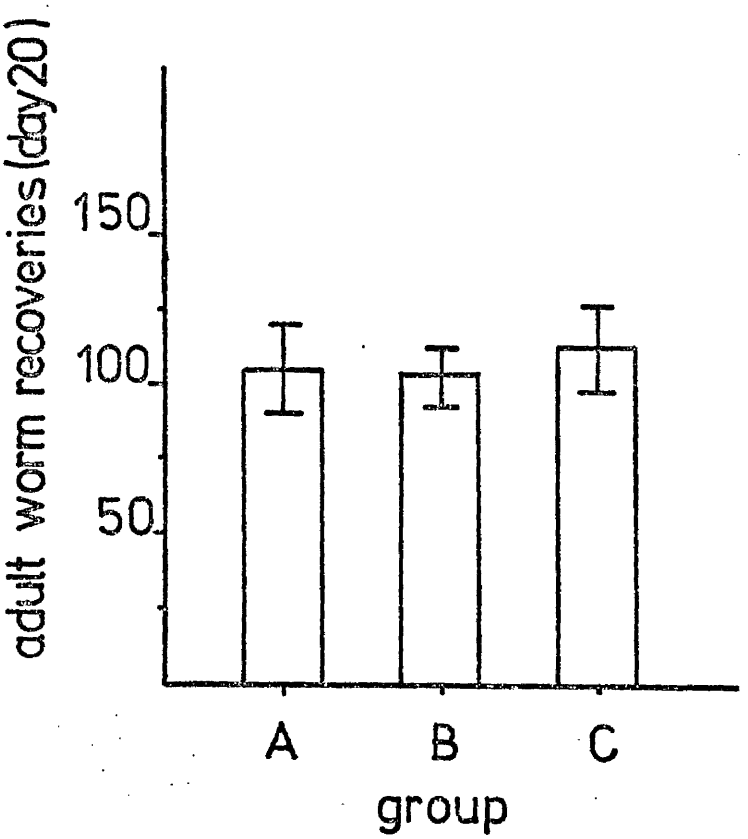
Worm recoveries (MWR $\pm$ SD) *N. dubius* day 20 post infection with 100 *N. dubius*

GROUP

A 100 *N. dubius* + Coumarin (25mg/kg daily)

B 100 *N. dubius* + 2% alcohol daily

C 100 *N. dubius*


adult worm recoveries (day 20)
150
100
50
A
B
C
group

Facing page 155

FIGURE 2-12

Egg counts (e.p.g. x 10^{-3}) from groups in Figure 2-11

GROUP

● 100 N. dubius + Coumarin

□ 100 N. dubius + 5% Alcohol

○ 100 N. dubius

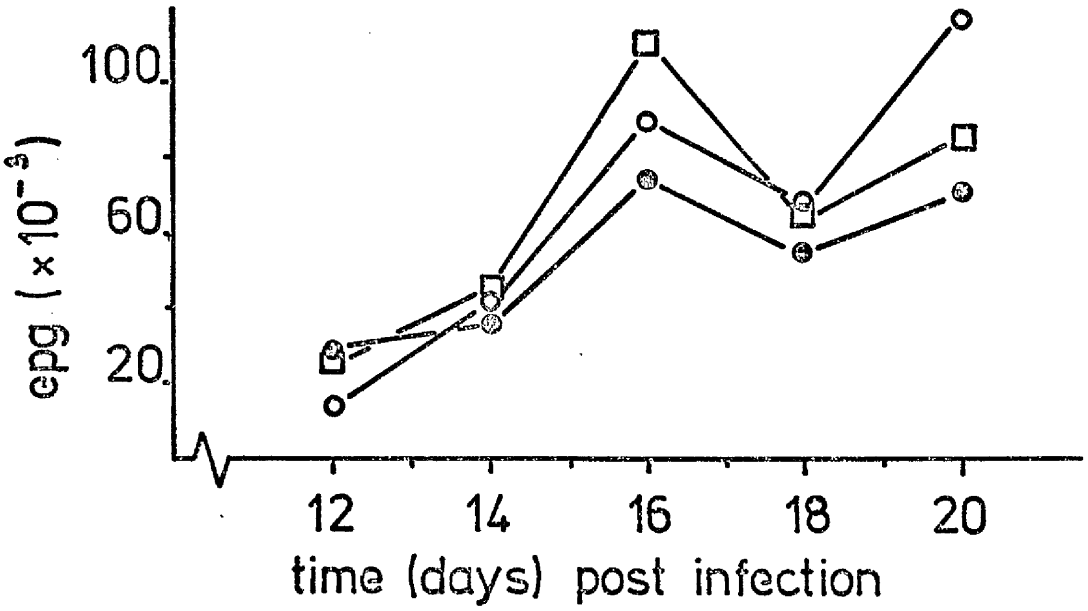

epg ($\times 10^{-3}$)
100
60
20
12
14
16
18
20
time (days) post infection

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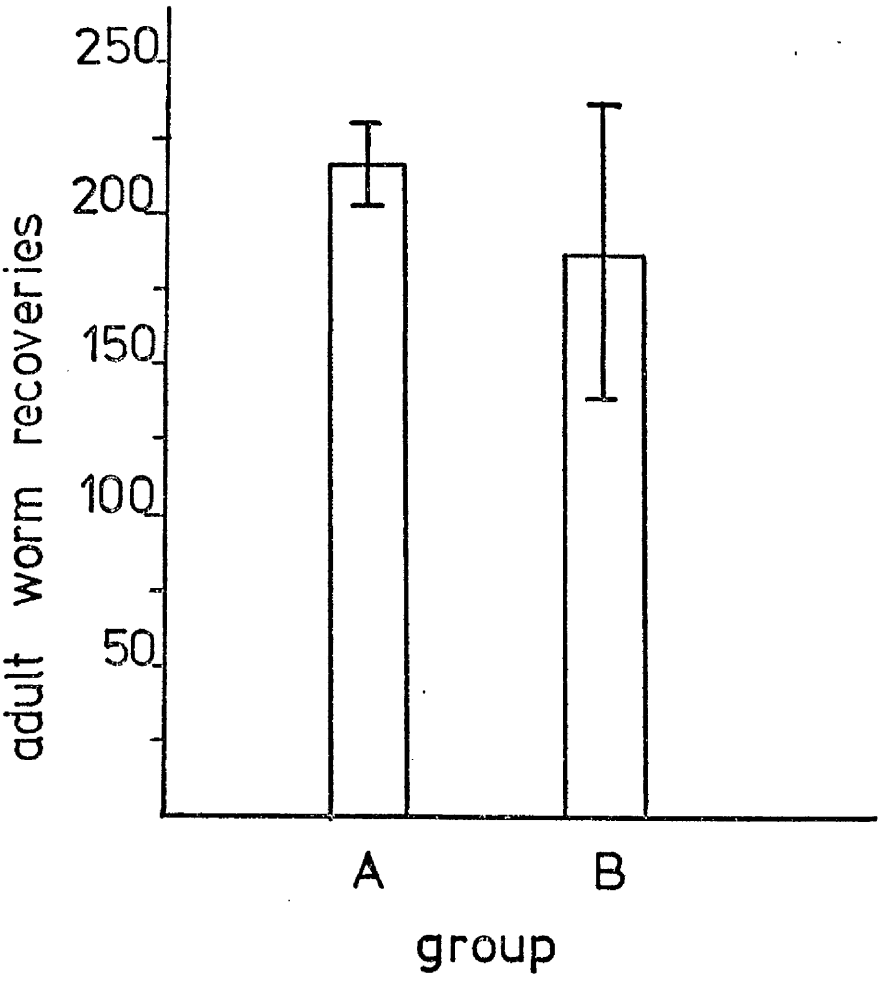
FIGURE 2-13

Effect of splenectomy on *N. dubius* infection

Worm recoveries (MWR ± SD) *N. dubius* day 20 post infection with 300 *N. dubius*

GROUP	
A	300 *N. dubius*
B	300 *N. dubius* splenectomised mice


250
200
150
100
50
adult worm recoveries
A
B
group

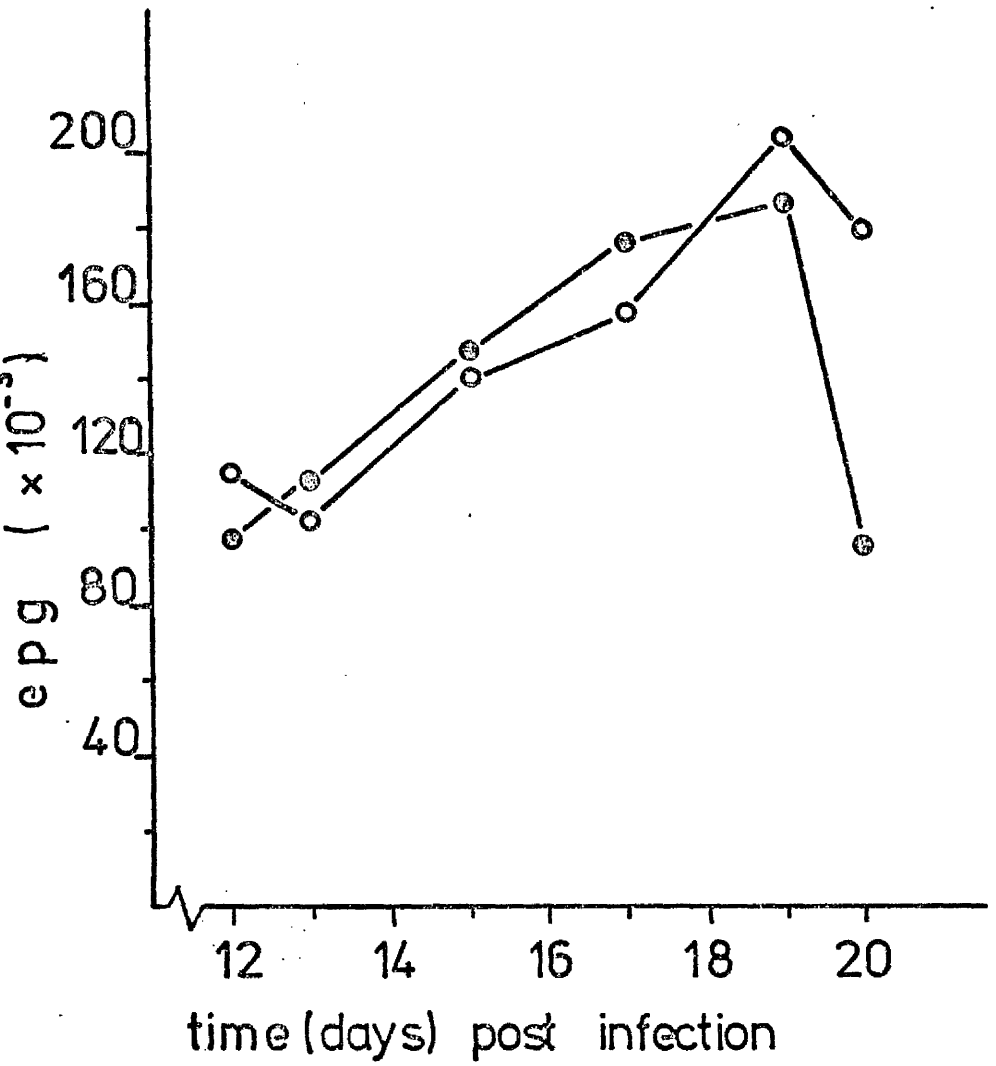
Facing page 157

FIGURE 2-14

Egg counts (e.p.g. x 10^{-3}) from groups in Figure 2-13

GROUP

- ○ 300 *N. dubius*
- ● 300 *N. dubius* splenectomised mice


200
160
120
80
40
epg (x 10⁻³)
12
14
16
18
20
time (days) post infection

PRIMARY AND SECONDARY INFECTION

In this experiment (Experiment 40) the effect of splenectomy upon a primary infection was re-examined and the effect upon a secondary infection was investigated. Eighteen male NIH mice were splenectomised and allowed 14 days post-operative recovery. These mice plus a group of age-matched controls were infected with 300 N. dubius on day 0. The egg counts from these groups were followed for 75 days post-infection, although only the first 35 days are shown (Figure 2-15). Six of the mice from each of these groups were killed on days 21 and 85 post-infection. Mean adult worm recoveries are shown in Figure 2-16. At day 35 of the primary infections two further groups, each of six mice, were treated with pyrantel (to remove adult worms) and these together with uninfected controls were subsequently challenged with 300 N. dubius on day 49. The remaining two groups were used to follow the egg counts of the primary infection until day 75.

The mean adult worm recoveries from the challenge infection groups, killed day 36 post-challenge are shown in Figure 2-17. Egg counts for the first 32 days of the challenge infections are shown in Figure 2-18.

There was some variability in the egg counts of the mice in the primary infections but at no time was there a difference between the groups (see Figure 2-15). The mean adult worm recoveries from the primary infections in splenectomised and control animals, day 21 and 85 were similar on both days although there was a slight drop in mean worm recovery, in both groups on day 85.

The egg counts from the challenge infection groups (Figure 2-18) differed from their controls (primary infection) in that they had

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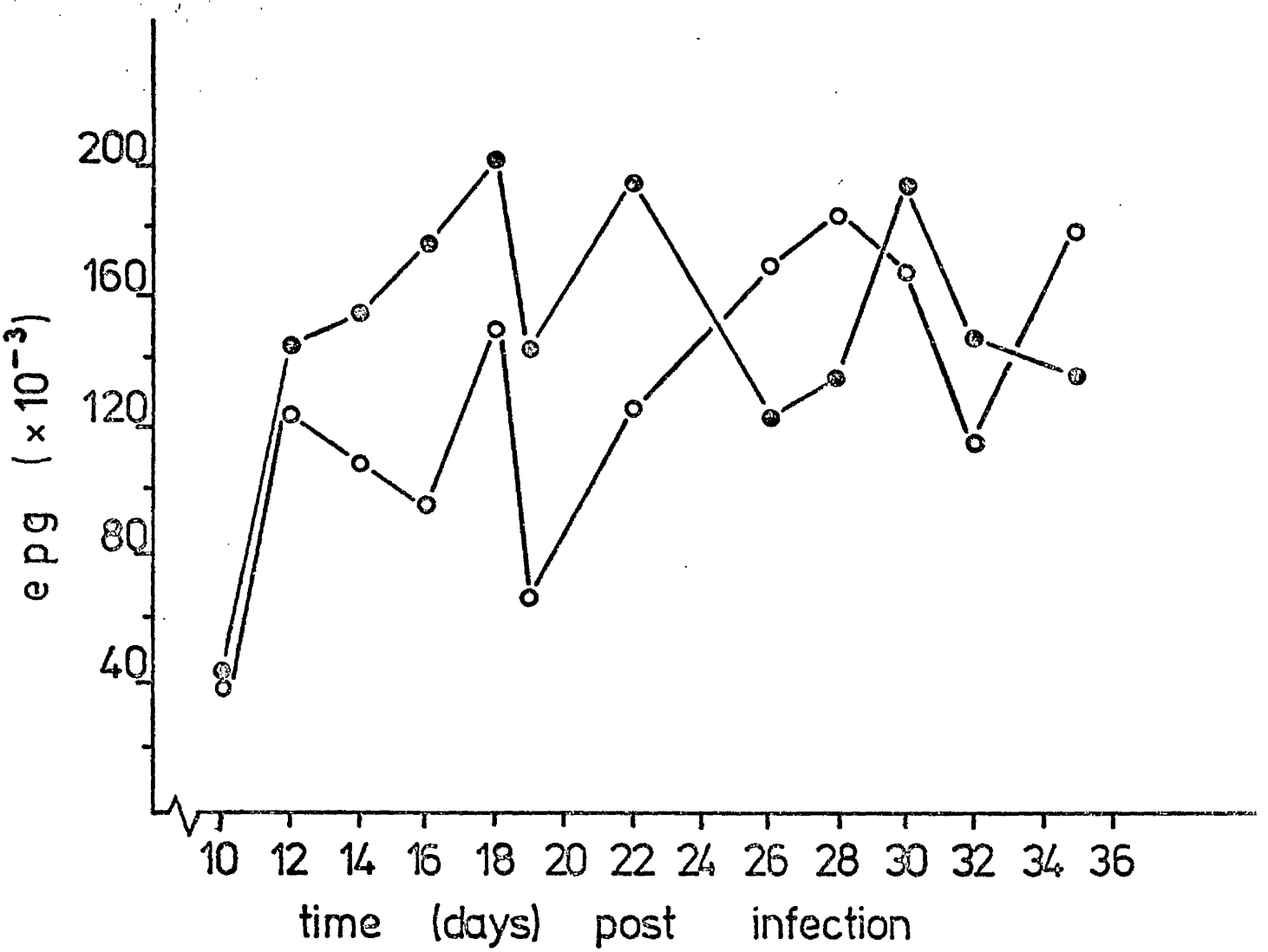
FIGURE 2-15

Effect of splenectomy on *N. dubius* infection

Egg counts (e.p.g. x 10^{-3})

○ 300 *N. dubius*

● 300 *N. dubius*, splenectomised mice


epg (x 10⁻³)
200
160
120
80
40
10 12 14 16 18 20 22 24 26 28 30 32 34 36
time (days) post infection

Facing page 160

FIGURE 2-16

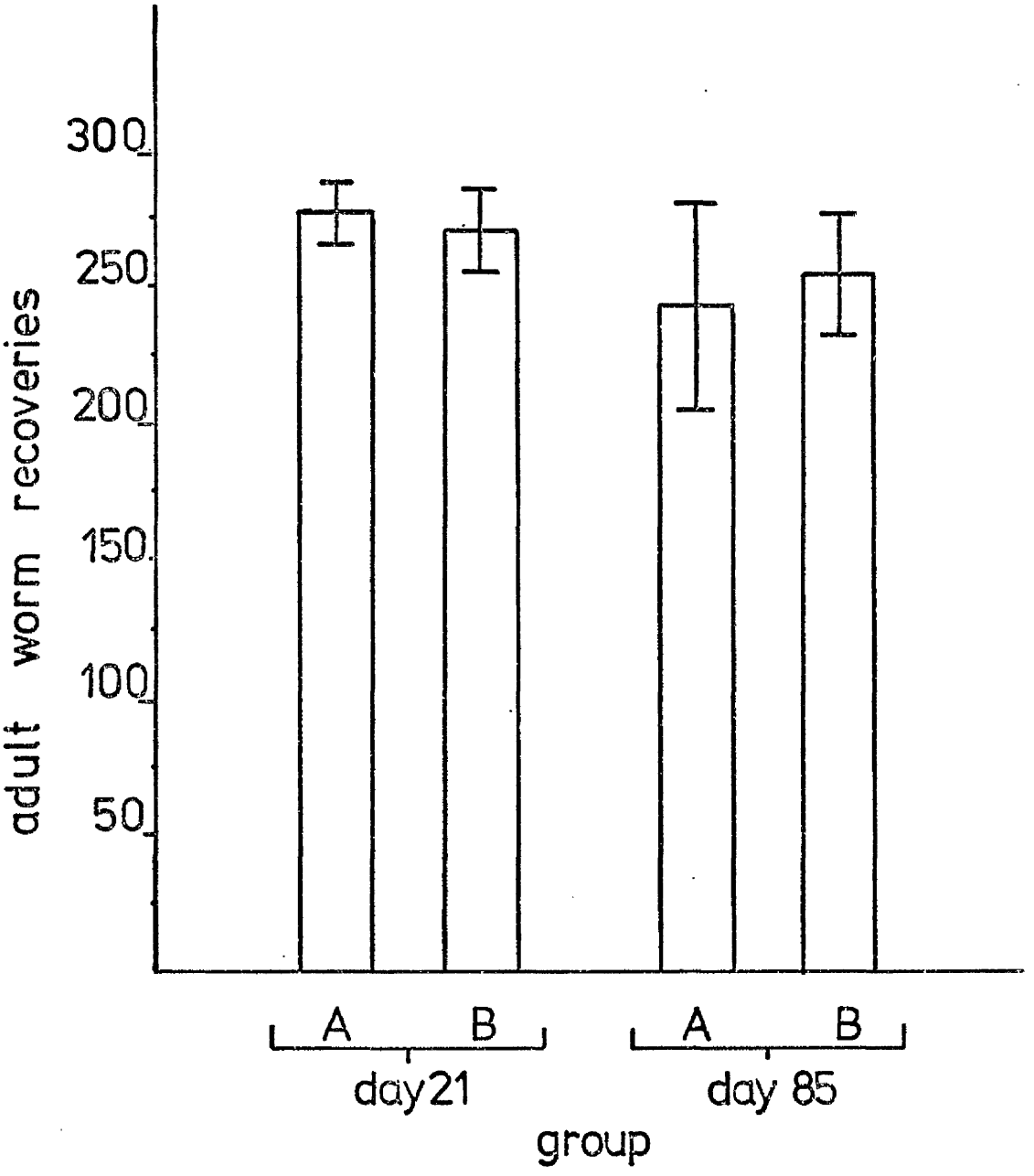
Effect of splenectomy on *N. dubius* infection

Mean recovery (MWR $\pm$ SD) *N. dubius* days 21 and 85 post infection with 300 *N. dubius*

GROUP

A 300 *N. dubius*

B 300 *N. dubius*, splenectomised mice


300
250
200
150
100
50
adult worm recoveries
A
B
A
B
day21
day 85
group

Facing page 161

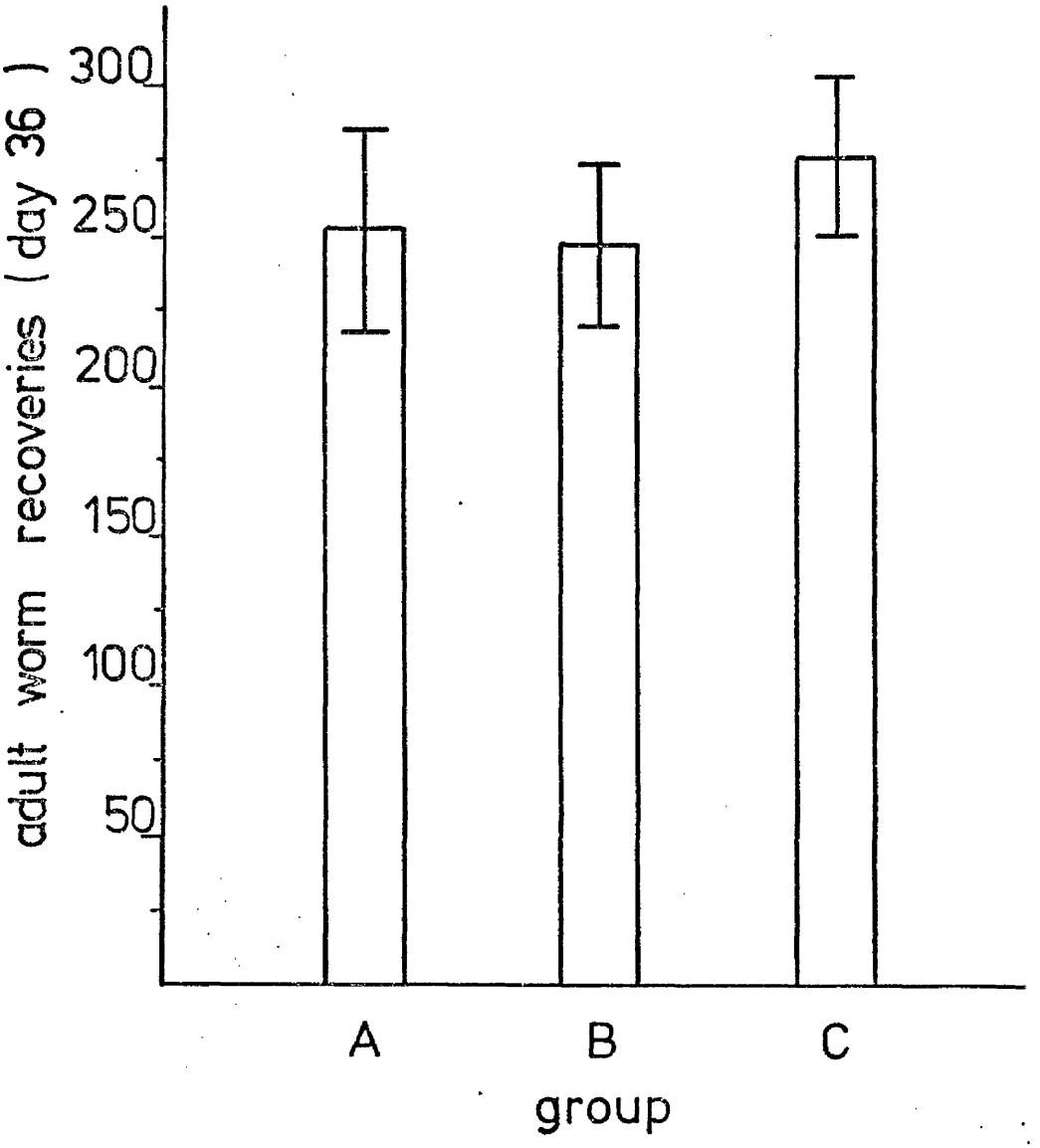
FIGURE 2-17

Effect of splenectomy on *N. dubius* infection

Mean recovery (MWR ± SD) *N. dubius*, day 36 post challenge with 300 *N. dubius*

GROUP

	1° infection	Challenge
A	300 *N. dubius*	300 *N. dubius* splenectomised mice
B	300 *N. dubius*	300 *N. dubius*
C	-	300 *N. dubius*


adult worm recoveries (day 36)
300
250
200
150
100
50
A
B
C
group

Facing page 162

FIGURE 2-18

Effect of splenectomy on *N. dubius* infection

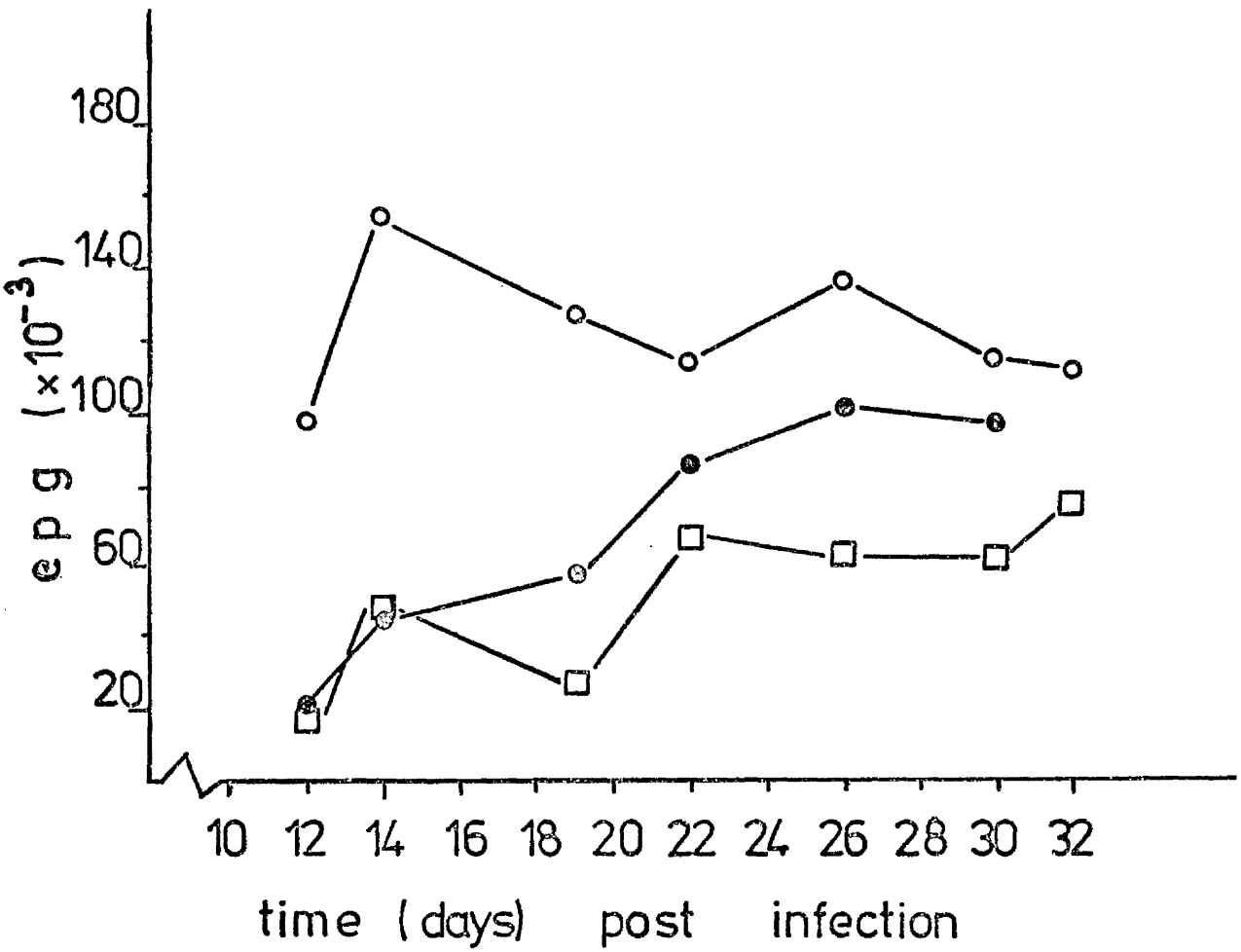
Egg counts (e.p.g. x 10^{-3}) from groups in Figure 2-17

GROUP

A ● challenge infection splenectomised mice

B □ challenge infection in normal mice

C ○ challenge control


180
140
100
60
20
epg (×10⁻³)
10 12 14 16 18 20 22 24 26 28 30 32
time (days) post infection

an initial, short term, low level of egg production. This occurred in both challenge infection groups and may have been due to delayed development of N. dubius. The mean adult worm recovery on day 36 post-challenge was 10.2% lower than controls in the challenged group, and 9.1% lower in the splenectomised/challenged group, but not significantly different from one another. These results indicate that there was no effect of splenectomy on the response of NIH mice to primary or challenge infection with N. dubius.

CAN ADULT N. DUBIUS BE EXPELLED FROM THE INTESTINE?

Previous attempts to stimulate immunity had failed to have much success in terms of the ability to transfer the response to naive recipients and no evidence had been obtained at any point to indicate that worms could be lost from the gut after they had matured to the adult stage. One possible explanation for the latter may be that N. dubius is exceptionally resistant to the action on inflammatory changes within the intestine. In order to exmaine the susceptibility of N. dubius to intestinal inflammation use was made of the inflammatory changes induced by T. spiralis infection. Adult N. dubius obtained from day 9 of a primary infection in donor mice were recovered and transplanted, 50 at a time, into the intestines of mice which had previously been infected with T. spiralis.

In the first experiment (Experiment 41) six male NIH mice were infected with 315 T. spiralis on day 0, six mice were infected with 300 N. dubius on the same day. Worms from the N. dubius infected mice were recovered from the intestine on day 9 and transferred, 50 at a time, by laparotomy to the small intestine of the six mice infected with T. spiralis and also to the small intestines of uninfected control mice. This worm transfer was at a time when the inflammatory response in the T. spiralis infected mice was maximal, as judged

macroscopically. Five days after transfer, when T. spiralis worms had been expelled from the gut the mice in both groups were killed and adult N. dubius were recovered. The results are shown in Figure 2-19. Although there was some variation within the groups the recovery of N. dubius from mice which had been infected with T. spiralis (Figure 2-19, Group A), was significantly lower than that from controls (Group B). In a subsequent experiment (Experiment 42) the same protocol was followed except that mice were killed one day after transplantation of N. dubius. The results are shown in Figure 2-20. On this occasion there was a slight drop in the mean worm recovery from the T. spiralis infected group but this was not statistically significant and suggests that N. dubius established equally well in both groups of mice.

Three further experiments were then carried out using the protocols set out below. Laparotomy at day 7 of T. spiralis infection is an extremely difficult process because of the inflamed, fluid-filled intestine so it was decided to bring forward the worm transplants so that the day 9 N. dubius were inserted into the intestines of mice on day 2 of the T. spiralis infection before the inflammation had substantially altered the gut.

GROUP	DAY				
	0	1	2	3	13
A	Tsp		Nd_{50}	K	
B			Nd_{50}	K	
C		Tsp		Nd_{50}	K
D				Nd_{50}	K

Groups A and B were killed one day post transfer of the N. dubius to assess worm establishment and Groups C and D were killed on day 13

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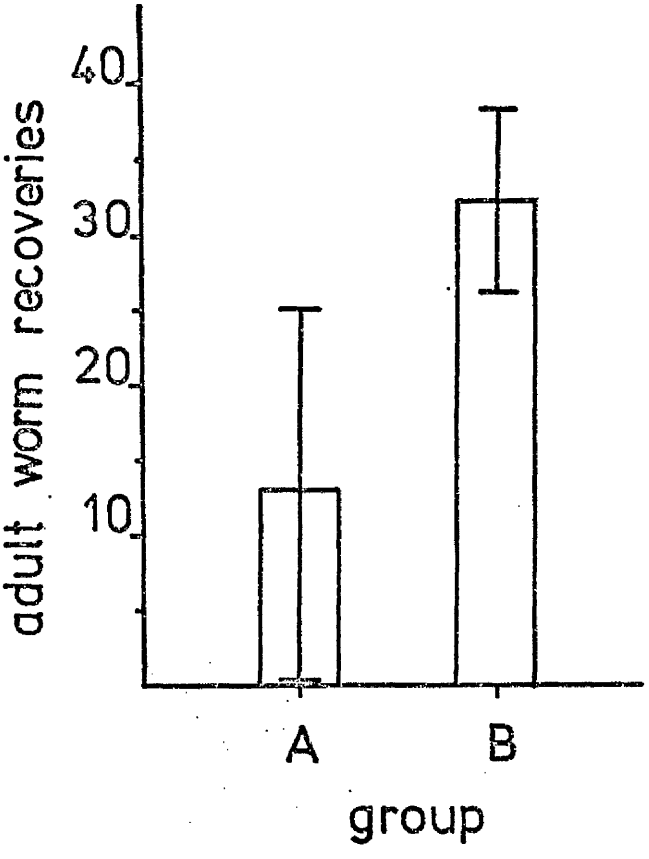
FIGURE 2-19

Survival of *N. dubius* (day 9) worms 5 days after transfer to *T. spiralis* infected mice

Mean recovery (MWR ± SD) *N. dubius*

Worms transferred on day 9 of *T. spiralis* infection

GROUP		
A	*T. spiralis* +	50 *N. dubius*
B	-	50 *N. dubius*


adult worm recoveries
40
30
20
10
A
B
group

Facing page 166

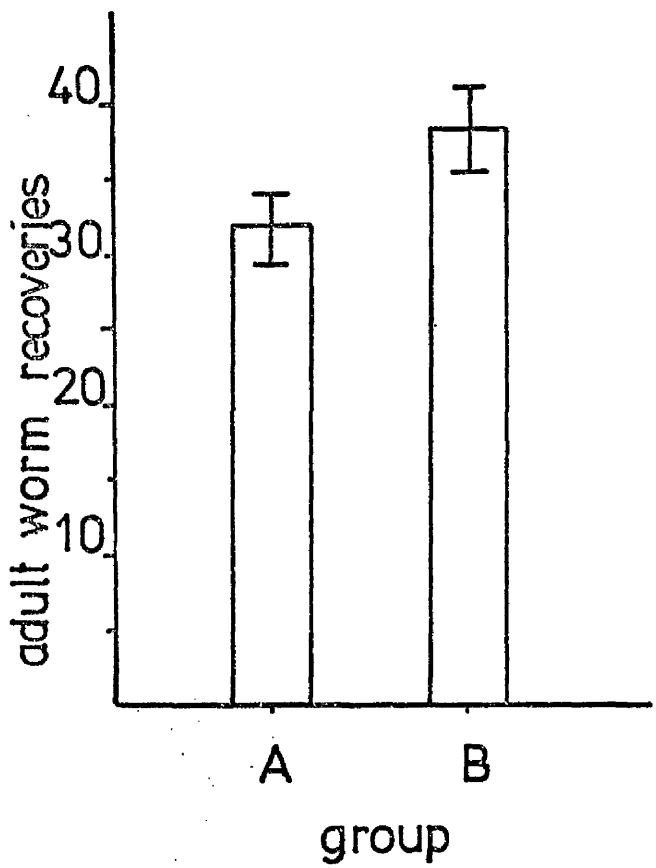
FIGURE 2-20

Survival of *N. dubius* (day 9) worms 1 day after transfer to *T. spiralis* infected mice

Mean recovery (MWR $\pm$ SD) *N. dubius*

Worms transferred on day 9 of *T. spiralis* infection

GROUP		
A	*T. spiralis* +	50 *N. dubius*
B	-	50 *N. dubius*


40
30
20
10
adult worm recoveries
A
B
group

when T. spiralis expulsion should have been all but complete. The results of the three experiments (Experiments 43, 44 and 45) are shown in Figures 2-21, 2-22 and 2-23 respectively. In each experiment, N. dubius establishment one day post transfer was the same in mice with and without T. spiralis infection. However in the groups killed on day 10 post transfer (day 12 of the T. spiralis infection) the worm recoveries were consistently and significantly lower in the groups which had harboured a T. spiralis infection. The results from all these experiments demonstrate clearly that under the correct intestinal conditions, adult N. dubius can be expelled from the small intestine. The conditions in this case were provided by the inflammation induced by T. spiralis but the results nevertheless show that mice can produce the changes necessary to expel N. dubius from the small intestine. Why they fail to do this during an initial N. dubius infection is the key question.

N. DUBIUS: FIELD STRAIN

In this section the response of NIH mice to the field strain of N. dubius isolated from Apodemus sylvaticus is described. Due to the difficulties involved in producing large numbers of infective larvae of field strain parasite only two short experiments were undertaken.

The first experiment (Experiment 46) examined the survival of field strain N. dubius in NIH mice. Eight mice were infected with 150 N. dubius (field) on day 0 and four of these were treated with cortisone acetate as described in General Materials and Methods; all mice were given antibiotics in the drinking water. Egg counts from the groups are shown in Figure 2-24.

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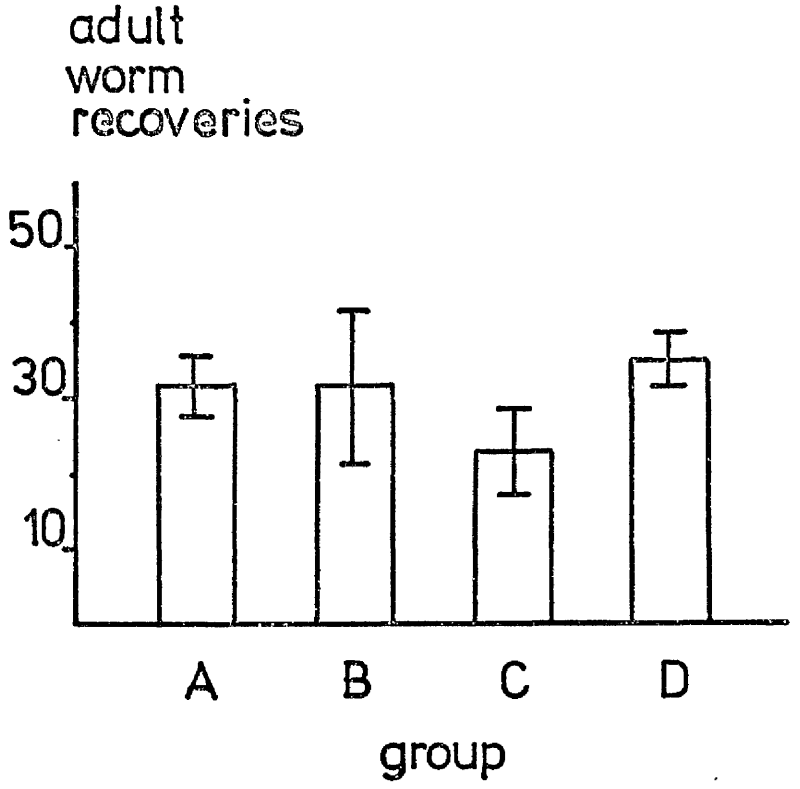
FIGURE 2-21

Survival of *N. dubius* (day 9) worms 1 day and 10 days after transfer to *T. spiralis* infected mice

Mean recovery (MWR $\pm$ SD) *N. dubius*

Worms transferred on day 2 of *T. spiralis* infection

GROUP	
A	*T. spiralis* + 50 *N. dubius* (1 day post transfer)
B	50 *N. dubius* (1 day post transfer)
C	*T. spiralis* + 50 *N. dubius* (10 days post transfer)
D	50 *N. dubius* (10 days post transfer)


adult
worm
recoveries
50
30
10
A
B
C
D
group

Facing page 169

FIGURE 2-22

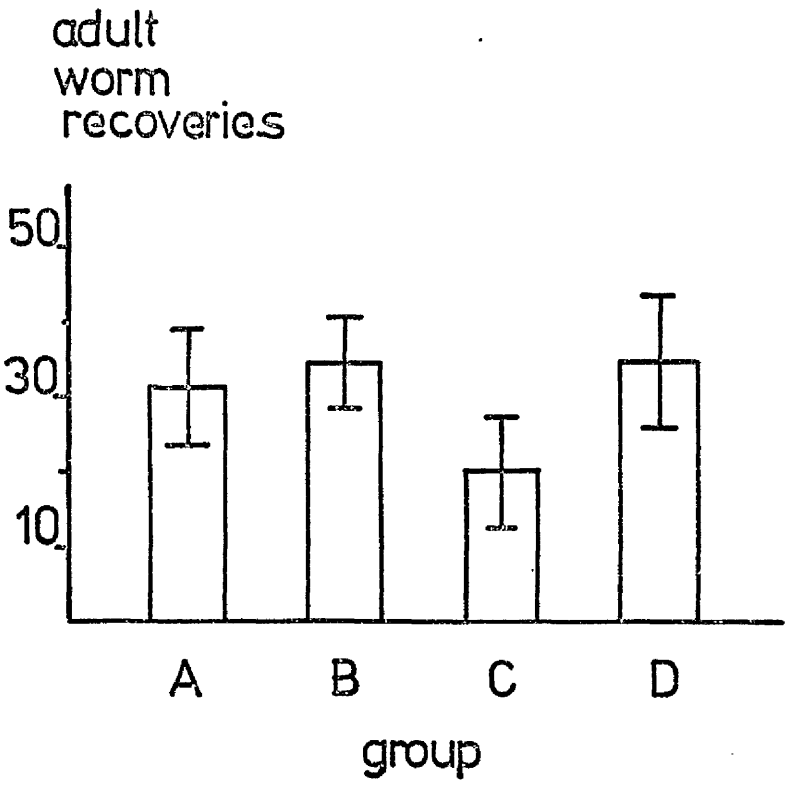
Survival of *N. dubius* (day 9) worms 1 day and 10 days after transfer to *T. spiralis* infected mice

Mean recovery (MWR $\pm$ SD) *N. dubius*

Worms transferred on day 2 of *T. spiralis* infection

GROUP

A	*T. spiralis* + 50 *N. dubius*	(1 day post transfer)
B	50 *N. dubius*	(1 day post transfer)
C	*T. spiralis* + 50 *N. dubius*	(10 days post transfer)
D	50 *N. dubius*	(10 days post transfer)


adult
worm
recoveries
50
30
10
A
B
C
D
group

Facing page 170

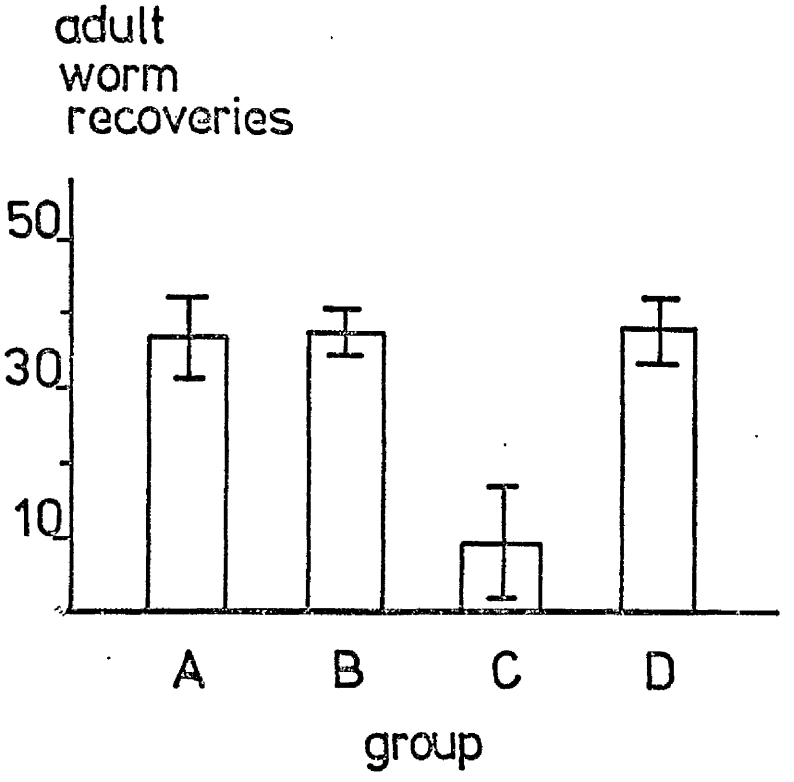
FIGURE 2-23

Survival of *N. dubius* (day 9) worms 1 day and 10 days after transfer to *T. spiralis* infected mice

Mean recovery (MWR $\pm$ SD) *N. dubius*

Worms transferred on day 2 of *T. spiralis* infection

GROUP	
A	*T. spiralis* + 50 *N. dubius* (1 day post transfer)
B	50 *N. dubius* (1 day post transfer)
C	*T. spiralis* + 50 *N. dubius* (10 days post transfer)
D	50 *N. dubius* (10 days post transfer)


adult
worm
recoveries
50
30
10
A
B
C
D
group

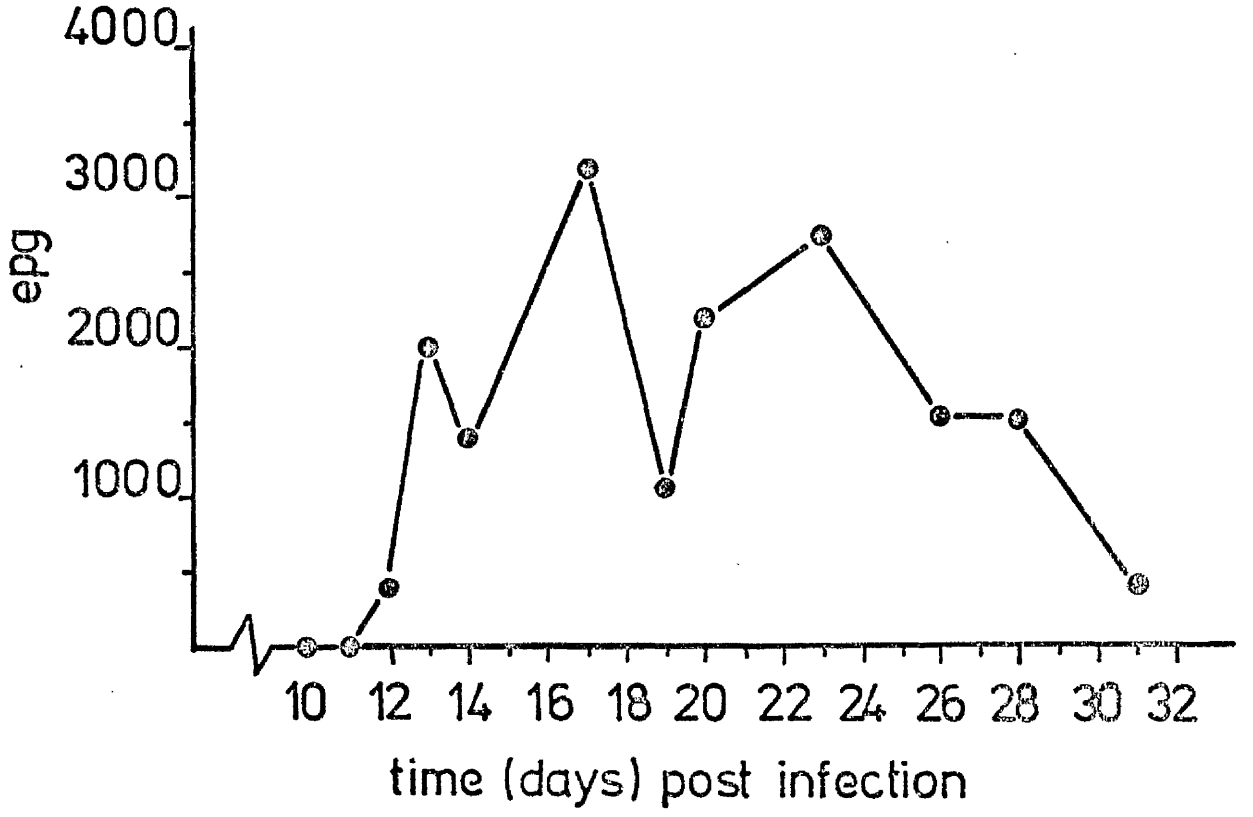
Facing page 171

FIGURE 2-24

Survival of N. dubius field strain in NIH mice

● 150 N. dubius (field) + CA

(150 N. dubius (field) no CA; no egg counts ever recorded).


4000
3000
2000
1000
epg
10 12 14 16 18 20 22 24 26 28 30 32
time (days) post infection

No egg counts or adult worms were ever recorded from mice not treated with cortisone acetate. The mice treated with cortisone acetate gave egg counts which rose until day 17 but then dropped off slowly, to day 12 values, by day 31. With such small numbers worm recoveries were difficult to interpret. There were 64 and 59 worms on day 24, and 54 and 0 worms on day 31. There appeared to have been some loss of worms from one of the mice but it is possible that the infection did not establish. The drop in egg counts which occurred after day 24, i.e. after the first mice were killed, could then be explained by the fact that only one of the remaining mice would have been passing eggs. However, against this interpretation is the positive finding of eggs in the faeces of each mouse when individual smears were prepared up until day 17 of infection.

In the second experiment in this section (Experiment 47) the original protocol was repeated but extended to compare the abilities of field strain and laboratory strain infections to immunize against challenge infection with the latter. The experimental design is outlined below:

GROUP	DAY				
	0 (10)	35	0 (20)	20	35
A	Nd (f)	K_5	Nd	K_3	K_4
B	Nd (f) + CA	K_5	Nd	K_6	K_6
C	Nd	K_5	Nd	K_5	K_6

Group A mice were infected with 100 N. dubius (field) and five were killed on day 35 of infection. Seven days later the remaining mice in this group were challenged with 100 N. dubius (laboratory). These mice had been treated with pyrantel on day 35 and were killed on days 20 and 35 post challenge. Group B mice were treated exactly as Group A except that they were given cortisone acetate every two days after

the primary infection. This was stopped on day 35 i.e. one week before the challenge infection with normal larvae. Group C were infected with 100 N. dubius on day 0, and five were killed on day 35. Following pyrantel treatment on day 35 the remaining mice were infected with 100 N. dubius on the same day as Groups A and B. The results are shown in Figure 2-25 (primary infection day 35 mean worm recoveries) Figure 2-26 (challenge infection laboratory strain mean worm recoveries, days 20 and 35) and Figure 2-27 (egg counts during the first 32 days of both primary and challenge infections). No worms were recovered from the field strain infection of the parasite in NIH mice (see Figure 2-25, Group A) on day 35 post infection. In Group B, the cortisone acetate treated group only two out of five mice had worms present at autopsy giving a low mean worm recovery for the group. The laboratory infection, Group C gave the expected high level of recovery at day 35. Egg counts for Group A were negative, by smear and flotation, throughout the experiment. The cortisone acetate treated group on the other hand (Group B) was positive for eggs and gave counts which remained at a very low level (Figure 2-27). There may be a number of reasons for this and these will be given in the discussion. The primary laboratory strain infection gave high egg counts throughout the experiment and despite the variability highlighted a major difference between laboratory and field strains. The challenge infection with the laboratory strain of the infection gave some interesting results (see Figures 2-26 and 2-27). The Group A mean worm recovery on both days 20 and 35 post challenge were lower than those of the field strain (cortisone acetate treated) and laboratory strain challenged (Group B) and the laboratory strain challenged with laboratory strain (Group C) infections. The reduction was 12-25% on day 20 post challenge but by day 35 had increased to 95%. The worm recoveries for the four mice killed on day 35 were 3, 5, 9

and 115; without the latter the group reduction would have been closer to 90%. The Group B worm recoveries were low on day 20, only 81% of control but by day 35 had reached control values (see Figure 2-26). This suggests a delay in the development of the parasite even though the mice in this group had been treated with cortisone acetate throughout the immunsing infection with the field strain. Group C gave the worm recoveries normally associated with a 100 x 100 laboratory infection challenge. The egg counts from the challenge infection were very interesting. Group A egg counts were always lower than those of Groups B and C and the differences were most pronounced towards the end of the infection, when it seems that some worms had been lost from this group (see Figures 2-26 and 2-27). As might have been expected the egg counts in Group B which were immunised under cortisone acetate were not as low as Group A and in fact remained similar to those of Group C, the laboratory x laboratory infection. The Group C egg counts were however lower than expected from mice with such a high (mean 99.5) worm burden but these low values can only be interpreted in terms of the effect of the host on the development and maturation of these worms as a result of the prior exposure to the laboratory strain of N. dubius. These results highlight the importance of parasite strain variations in the study of parasite elimination by the host and also suggest that the chronicity of an N. dubius laboratory infection in laboratory mice may be the end result of a gradual selection process; a process which has selected for a worm population which is relatively resistant to the immune response of the host.

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FIGURE 2-25

Survival of N. dubius field strain in NIH mice

(Immunizing abilities of field and laboratory strain infections)

Mean recovery (MWR $\pm$ SD) N. dubius day 35 post infection

GROUP	
A	100 N. dubius (field)
B	100 N. dubius (field) + CA
C	100 N. dubius (laboratory)

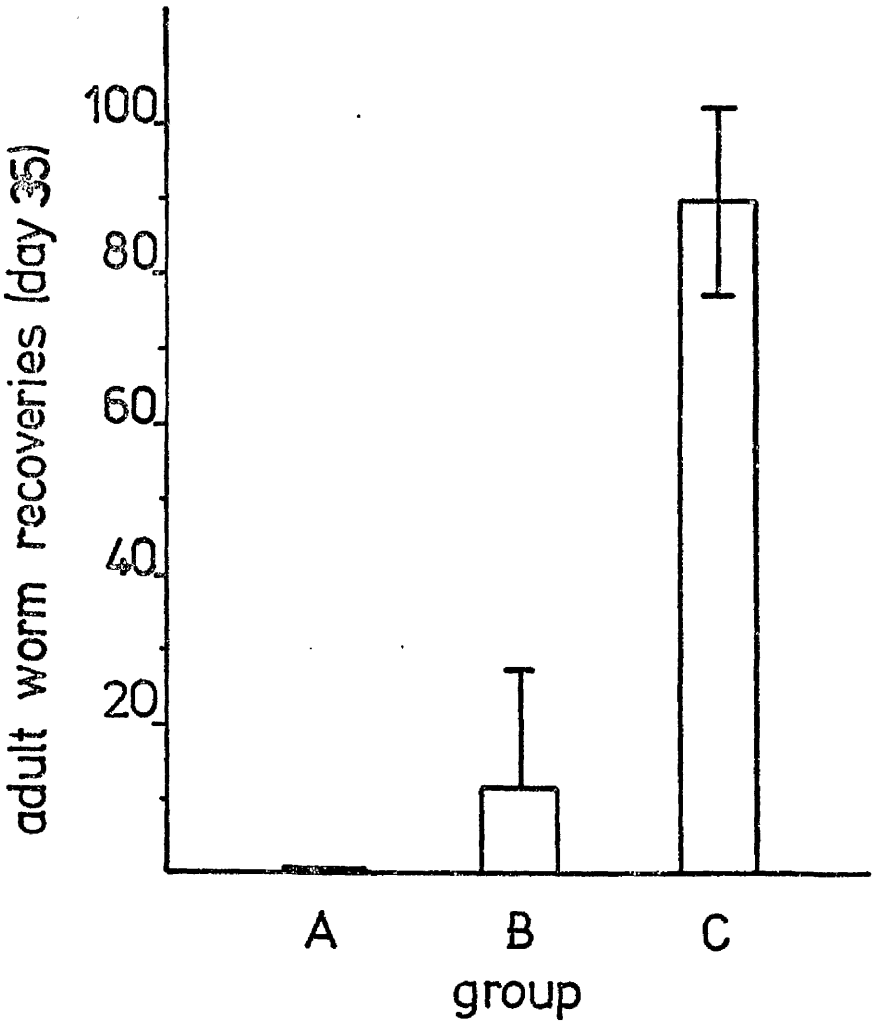
adult worm recoveries (day 35)
100
80
60
40
20
A
B
C
group

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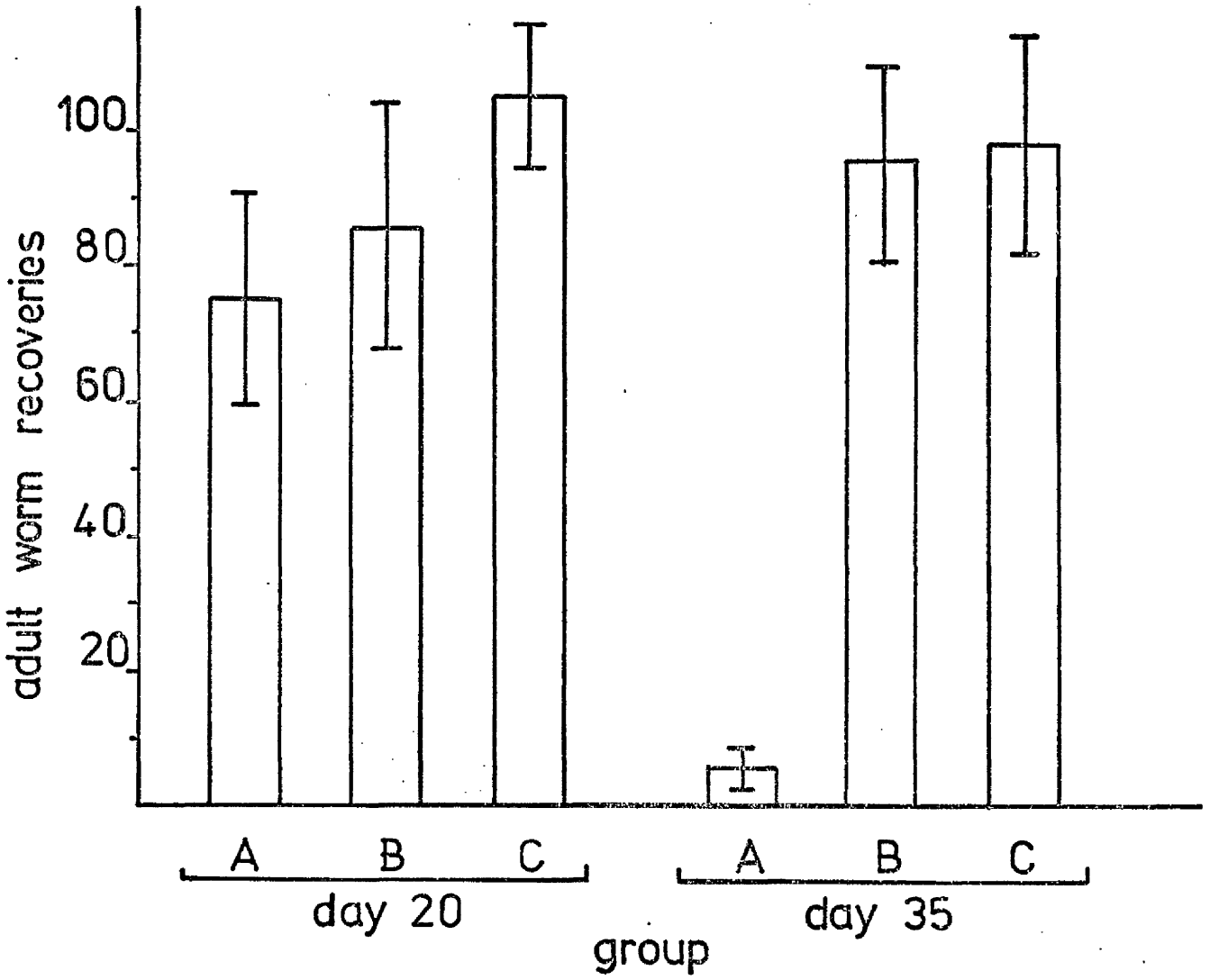
FIGURE 2-26

Survival of N. dubius laboratory strain in NIH mice

(Immunizing abilities of field and laboratory strain infections)

Mean recovery (MWR $\pm$ SD) N. dubius days 20, 35 post challenge 100 N. dubius (laboratory)

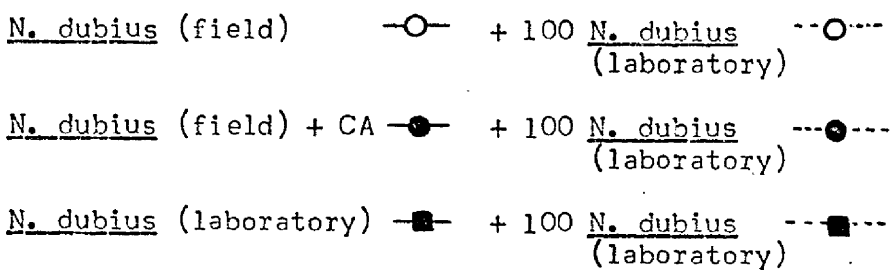
GROUP		
A	100 N. dubius (field)	+ 100 N. dubius (laboratory)
B	100 N. dubius (field) + CA	+ 100 N. dubius (laboratory)
C	100 N. dubius (laboratory)	+ 100 N. dubius (laboratory)

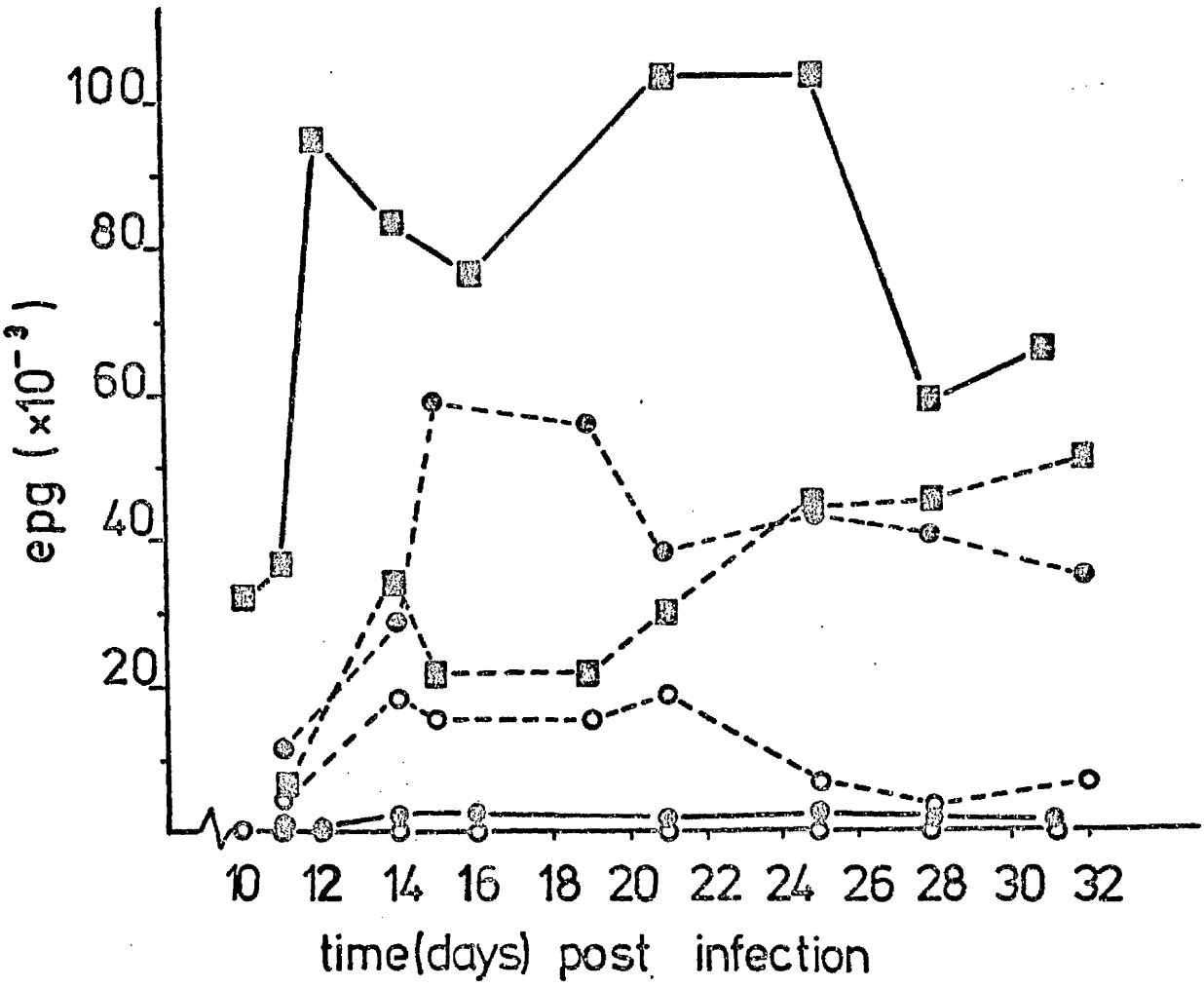

adult worm recoveries
100
80
60
40
20
A
B
C
A
B
C
day 20
day 35
group

Facing page 177

FIGURE 2-27

Egg counts (e.p.g. x 10^{-3}) from groups shown in Figures 2-25, 2-26.




100
80
60
40
20
epg (x10⁻³)
10 12 14 16 18 20 22 24 26 28 30 32
time(days) post infection

IRRADIATION AND N. DUBIUS

The final section of this thesis deals with the effect of irradiation on the growth, survival and reproduction of N. dubius and subsequently with the use of irradiated parasites in stimulating immunity. Part of the work is included in the form of a paper prepared in conjunction with Dr J M Behnke and Miss Heather A Parish of Nottingham University. The paper has been accepted for publication in Parasite Immunology.

EFFECT OF IRRADIATION AT 6.5 K.RADS.

In the first experiment (Experiment 48) parasites were exposed to 6.5 K.rads to assess the effect of irradiation on growth and development. This dose was chosen as previous reports of the effect of irradiation on nematode parasites have shown that at doses of about 6 K.rads the egg/larvae production by female worms is dramatically altered. The dose was given to a batch of larvae as described in the materials and methods of this section. Twelve male NIH mice were divided into 2 groups, 6 receiving an infection of 100 normal larvae and 6 receiving 100, 6.5 K.rads irradiated larvae. Both these groups were killed on day 20 for worm recoveries and both had egg counts taken periodically during the infection. The mean worm recoveries are shown in Figure 2-28 and egg counts in Figure 2-29. The Cobalt 60 irradiation had caused a significant drop in the mean worm recovery from Group B when compared to control, Group A. The egg counts from Group B did not reach detectable levels until day 14 whereas control animals in Group A were passing large numbers of eggs by day 12. The maximum egg count from Group B was less than one tenth of that from Group A.

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FIGURE 2-28

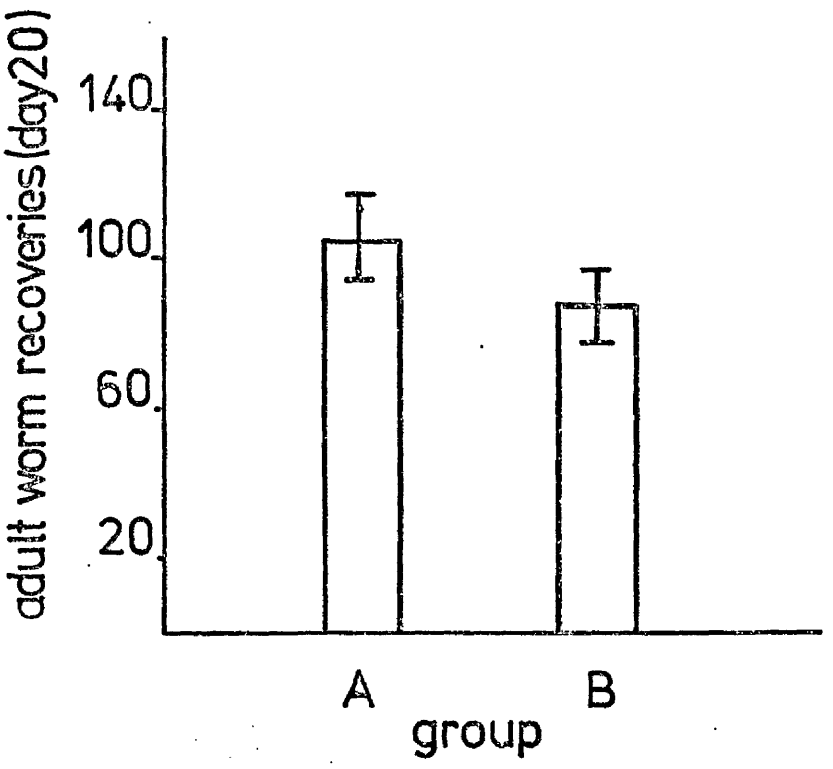
Effect of Irradiation at 6.5 K.rads on N. dubius infection

Mean recoveries (MWR ± SD) N. dubius on day 20 post infection with 100 N. dubius

GROUP

A 100 N. dubius normal larvae

B 100 N. dubius irradiated 6.5 K.rads

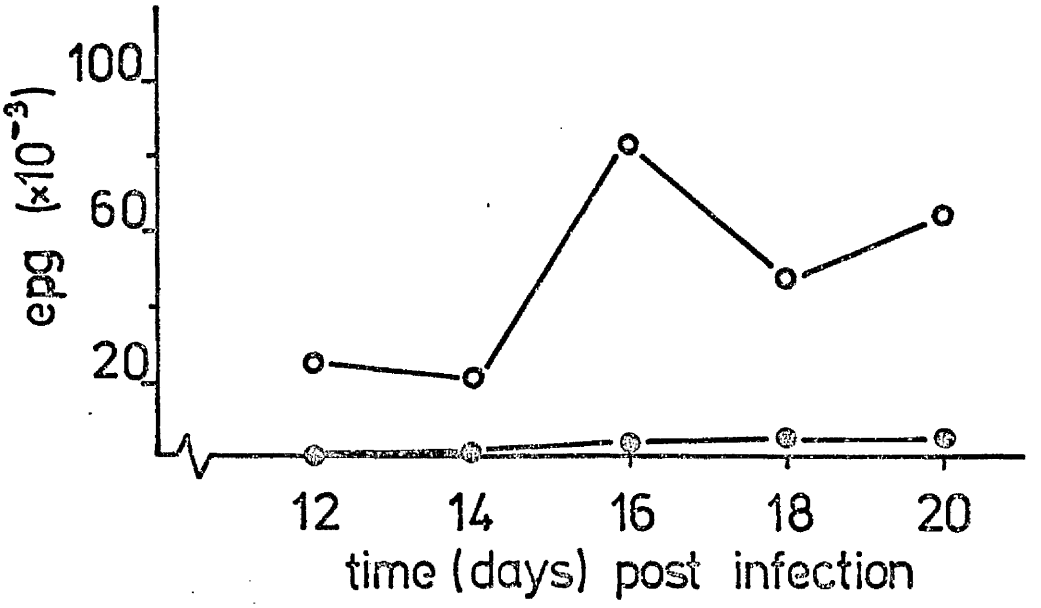

adult worm recoveries (day20)
140
100
60
20
A
B
group

Facing page 180

FIGURE 2-29

Egg counts (e.p.g. x 10^{-3}) from groups shown in Figure 2-28

- O 100 N. dubius normal larvae
- ● 100 N. dubius irradiated 6.5 K.rads


epg (×10⁻³)
100
60
20
12
14
16
18
20
time (days) post infection

EFFECT OF VARIOUS DOSES OF COBALT 60 IRRADIATION ON N. DUBIUS

The previous experiment had shown that Cobalt 60 irradiation of larvae did have an effect on their subsequent survival in the host and on their reproductive potential. To extend the information, two experiments were set up to examine the effects of irradiation at other levels.

In the first experiment (Experiment 49) there were 24 mice (male NIH) in each group and each mouse was infected with 100 N. dubius which had irradiated at the specified levels (2.5 K.rad, 7.5 K.rad, 15 K.rad, 25 K.rad and unirradiated controls). Six mice from each group were killed on days 10, 14, 21 and 35 post infection. In the second experiment (Experiment 50) there were only 12 mice (male NIH) in each group and these were infected as before and killed on days 14 and 35 post infection. The results are shown in Figure 2-30 (worm recoveries on days 10, 14, 21 and 35, Experiment 49), Figure 2-31 (egg counts) and in Figure 2-32 (worm recoveries on days 14 and 35, Experiment 50).

In Experiment 49 the control N. dubius recoveries were lower than expected but since the same batch of larvae was used for the irradiated groups the infectivity does not present any problems for the interpretation of the results. In all groups, regardless of the level of irradiation used, the worm recoveries remained approximately constant throughout the experiment i.e. there was no evidence for a gradual loss of worms after day 14. Only the 15 K.rad and 25 K.rad showed any decrease in the mean worm recovery and these two groups remained below the control values. The lowest level of irradiation, 2.5 K.rad, seemed to enhance the establishment of the larvae; in this group recoveries were significantly higher than those of the controls on all days except day 21. Irradiation at 2.5 K.rad had no

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FIGURE 2-30

Effect of irradiation at 0, 2.5, 7.5, 15 and 25 K.rad on infection with N. dubius

Mean recoveries (MWR $\pm$ SD) N. dubius at days 10, 14, 21 and 35 post infection with 100 larvae

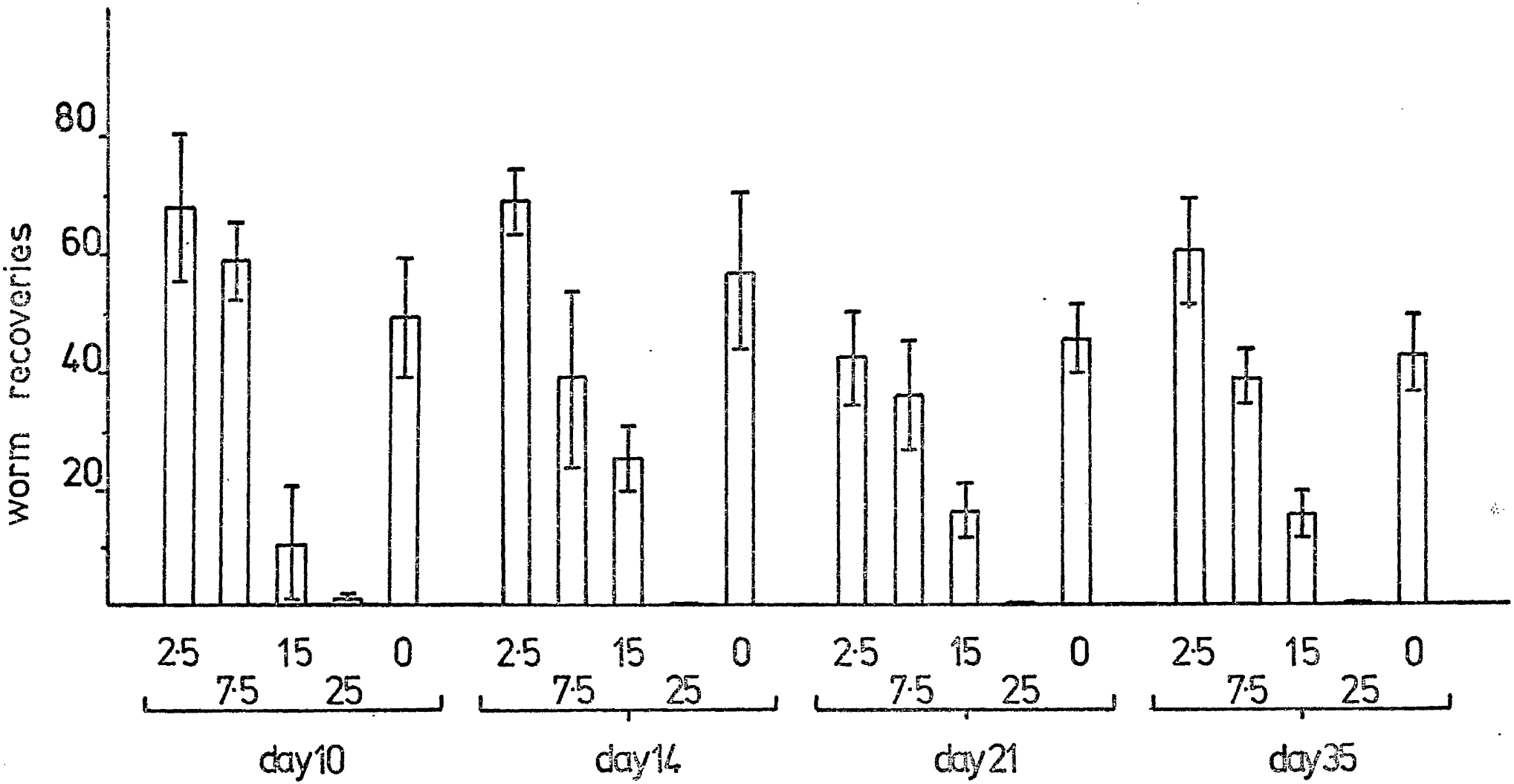
worm recoveries
80
60
40
20
2·5
7·5
15
25
0
day10
day14
day21
day35

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FIGURE 2-31

Effect of irradiation at 0, 2.5, 7.5, 15 and 25 K.rads on egg counts (e.p.g. x 10^{-3}) from N. dubius infected mice shown in Figure 2-30

- ○ 100 N. dubius normal larvae
- ● 100 N. dubius 2.5 K.rad irradiated larvae
- □ 100 N. dubius 7.5 K.rad irradiated larvae

No egg counts were recorded from mice given infections with 15 and 25 k.rad irradiated larvae.

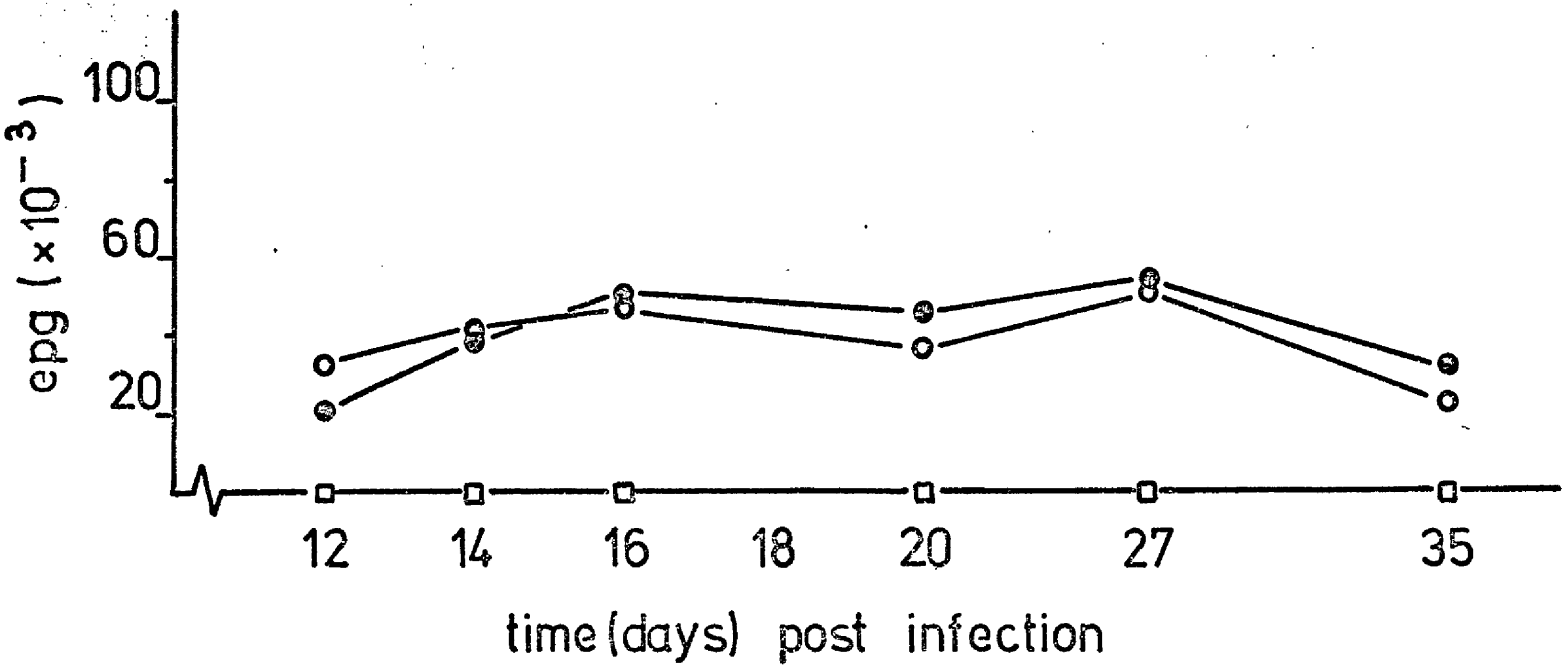
epg (×10⁻³)
100
60
20
12
14
16
18
20
27
35
time (days) post infection

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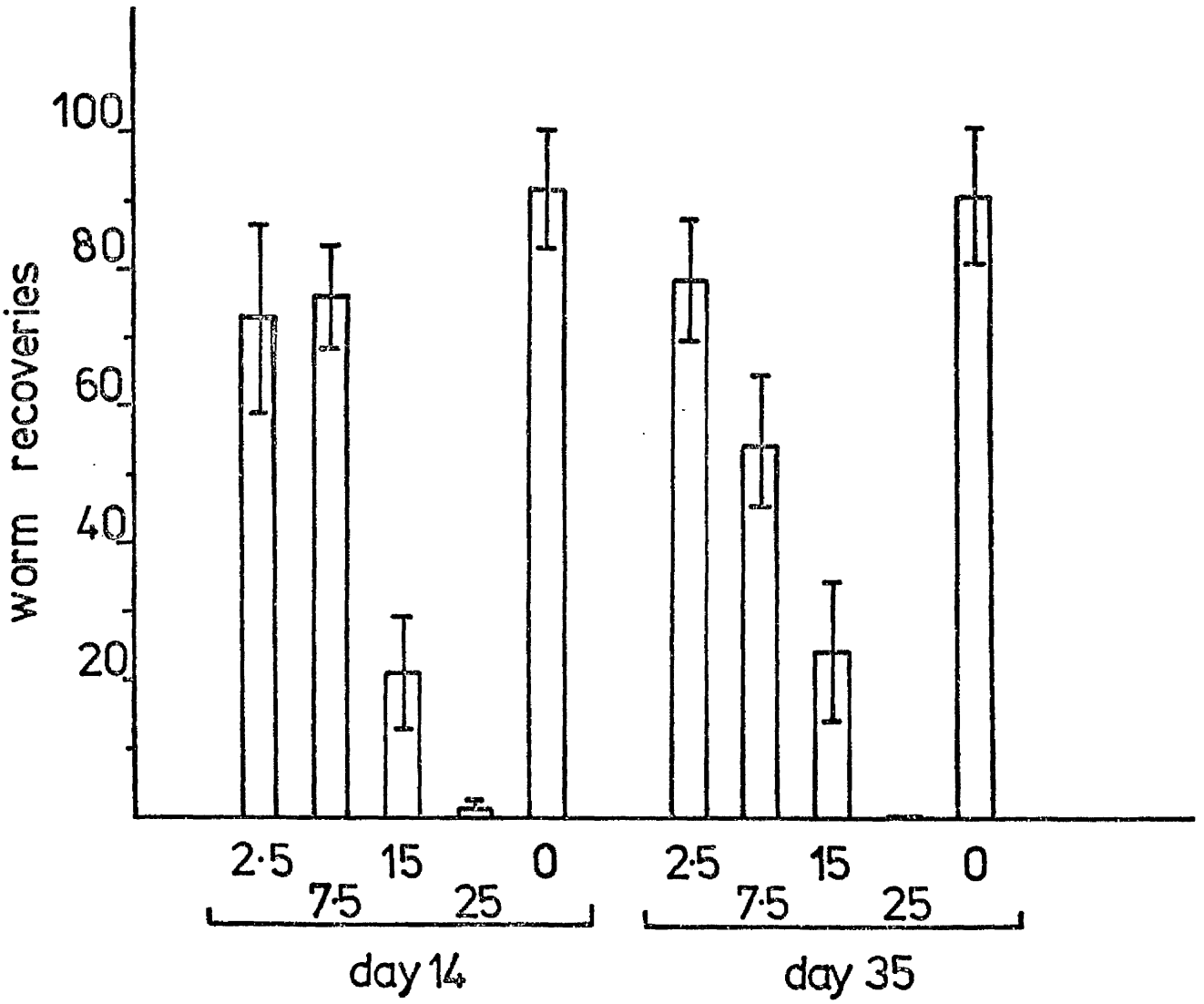
FIGURE 2-32

Effect of irradiation at 0, 2.5, 7.5, 15 and 25 K.rads on infection with *N. dubius*

Mean recoveries (MWR $\pm$ SD) *N. dubius* at 14 and 35 days post infection with 100 larvae


worm recoveries
100
80
60
40
20
2·5
7·5
15
25
0
2·5
7·5
15
25
0
day 14
day 35

detectable effect on egg counts (see Figure 2-31) and mice in this group gave similar values to those of controls. In keeping with the previous result (at 6.5 K.rad), irradiation at 7.5 K.rad completely abolished egg production from female worms. No egg production was ever recorded for the 15 K.rad and 25 K.rad irradiated groups. In the second experiment (Experiment 50) mean worm recoveries on days 14 and 35 of infection followed the pattern set in the previous experiment (see Figure 2-32). No egg counts were made. As in the previous experiment low levels of irradiation, (2.5 K.rad and 7.5 K.rad) had little effect on worm survival but above 10 K.rad a marked reduction was evident.

IS THE REDUCTION IN WORM NUMBERS DUE TO IRRADIATION DAMAGE OR TO A HOST IMMUNE RESPONSE?

To answer this question an experiment (Experiment 51) was set up using the experimental design set out below:

GROUP	DAY		
	0	+14	+21
A	100 Nd ↯ 15 K.rad	CA CA CA	K_6
B	100 Nd ↯ 15 K.rad	K_6	K_6
C	100 Nd	K_6	K_6

Six week old male NIH mice, were infected with 100 normal N. dubius or with 100 N. dubius which had been irradiated at 15 K.rad (100 Nd ↯ 15 K.rad). Group A mice, which were infected with irradiated larvae were given cortisone acetate on days 14, 16 and 18 to suppress the tissue response against the parasites. The mice were killed as shown and the mean adult worm recoveries are shown in Figure 2-33. The day 14 kill of Groups B and C gave the initial establishment levels of 15 K.rad and normal larvae respectively. These results agree with those of the previous experiment and show

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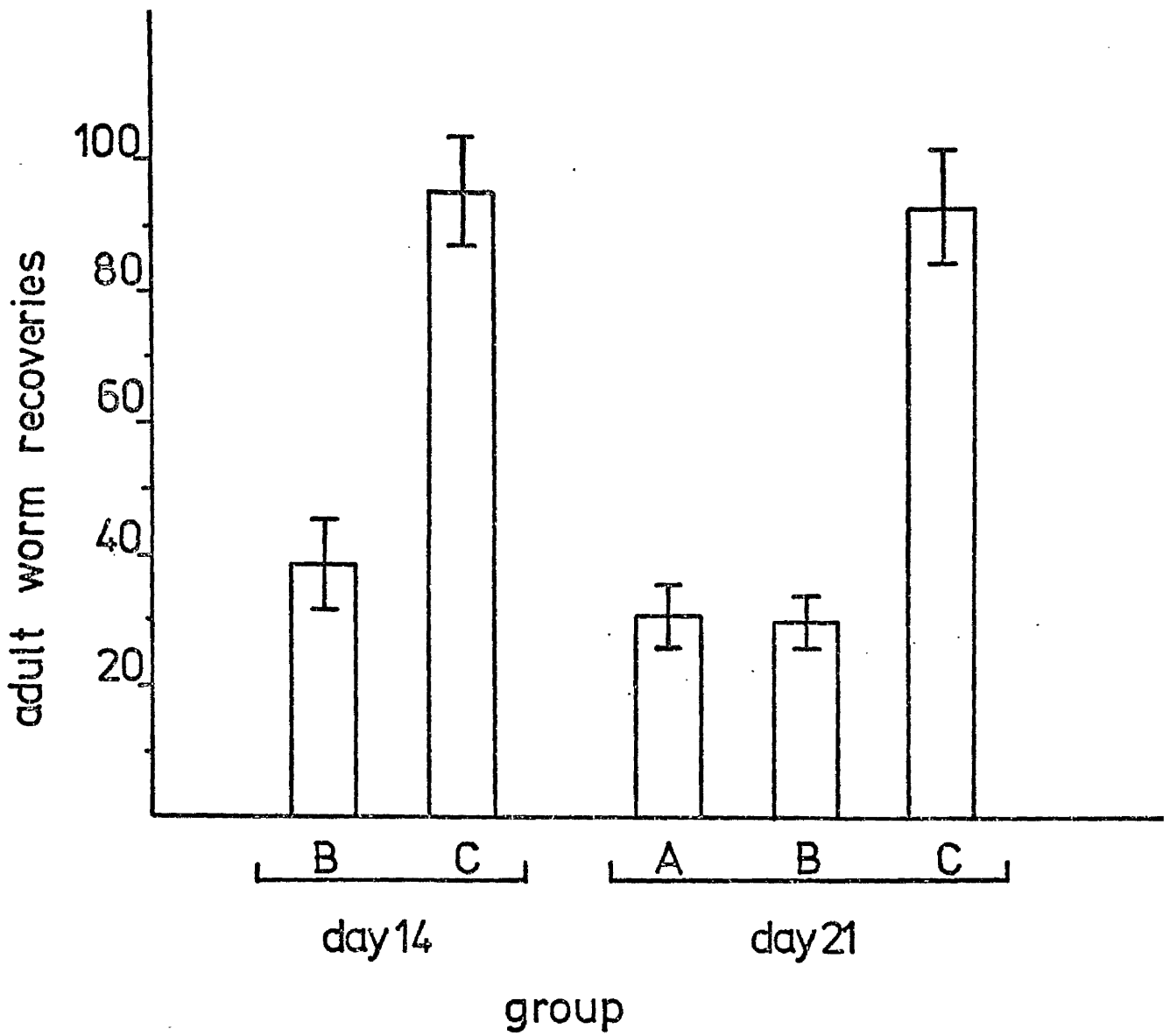
FIGURE 2-33

Effect of irradiation at 15 K.rads on infection with N. dubius

Is the reduction in worm numbers due to irradiation damage or a host immune response?

Mean recovery (MWR $\pm$ SD) N. dubius at days 14 and 21 post infection with 100 N. dubius

GROUP	DAY		
	0	+14	+21
A	100 Nd (15 K.rad)	CA CA CA	K_6
B	100 Nd (15 K.rad)	K_6	K_6
C	100 Nd	K_6	K_6


adult worm recoveries
100
80
60
40
20
B
C
A
B
C
day 14
day 21
group

that the establishment of adult worms from irradiated larvae was markedly reduced compared to controls. The results for day 21 show that cortisone acetate treatment did not alter the numbers of parasites recovered from mice infected with irradiated larvae. This suggests that the decreased recovery of worms is due to the initial irradiation damage and not to a host immune response. Groups A and B gave similar results and both were significantly lower than controls. This experiment would have been better controlled by having a 15 k.rad group treated with cortisone acetate from the beginning of the infection.

EFFECT OF IRRADIATION ON THE IMMUNOSUPPRESSIVE ACTION OF N. DUBIUS

The results from the previous experiments indicate that irradiation with Cobalt 60 can have a dramatic effect on N. dubius. Before examining the use of irradiated larvae in stimulating immunity the immunosuppressive action of irradiated parasites was examined. Once again T. spiralis expulsion was used as a measure of the suppressive effect. The experimental design (Experiment 52) is set out below:

GROUP		DAY		
	0	8	12	16
A	300 Nd + Tsp	K_6	K_6	K_6
B	300 Nd (7.5 K.rad) + Tsp	-	K_6	K_6
C	300 Nd (15 K.rad) + Tsp	-	K_6	K_6
D	1500 Nd (15 K.rad) + Tsp	K_3	K_3	-
E	300 Nd	-	K_3	K_3
F	Tsp	K_6	K_6	K_6

Group D were given 1500 N. dubius (15 K.rad) in an attempt to increase the number of parasites which would survive to the lumen stage and thus to compensate for the irradiation damage to the parasite. However, this level of infection was found to be more than the mice could tolerate

and so all of the mice in the group were killed by day 12. T. spiralis infections were with 300 larvae throughout. T. spiralis mean adult worm recoveries are shown in Figure 2-34. N. dubius worm recoveries are not shown but they were similar (relatively) to those obtained in earlier experiments using irradiated larvae. The day 8 kills were made to check the establishment of T. spiralis in N. dubius infected (Group A) mice and in uninfected control (Group F) mice. The T. spiralis recoveries in Groups A and F were similar on day 8 (see Figure 2-34). By day 12 almost all the T. spiralis had been expelled from the controls (Group F) and no T. spiralis were recovered on day 16. Group A T. spiralis mean worm recovery was higher than would normally be recovered from a 12 day old infection (compare control Group F) and followed the pattern set in Experiments 1 and 2. The day 16 mean worm recovery for Group A was lower than that for day 12 suggesting a gradual loss of adult worms. As before infection with 300 N. dubius delayed the expulsion of the concurrent T. spiralis infection. The other groups, Groups B, C and D showed the delayed expulsion pattern of Group A rather than the normal expulsion pattern of Group F (Figure 2-34). So it appears that even with the degree of irradiation damage caused by 15 K.rad. the suppression caused by N. dubius still operates against the mechanisms involved in resistance against T. spiralis.

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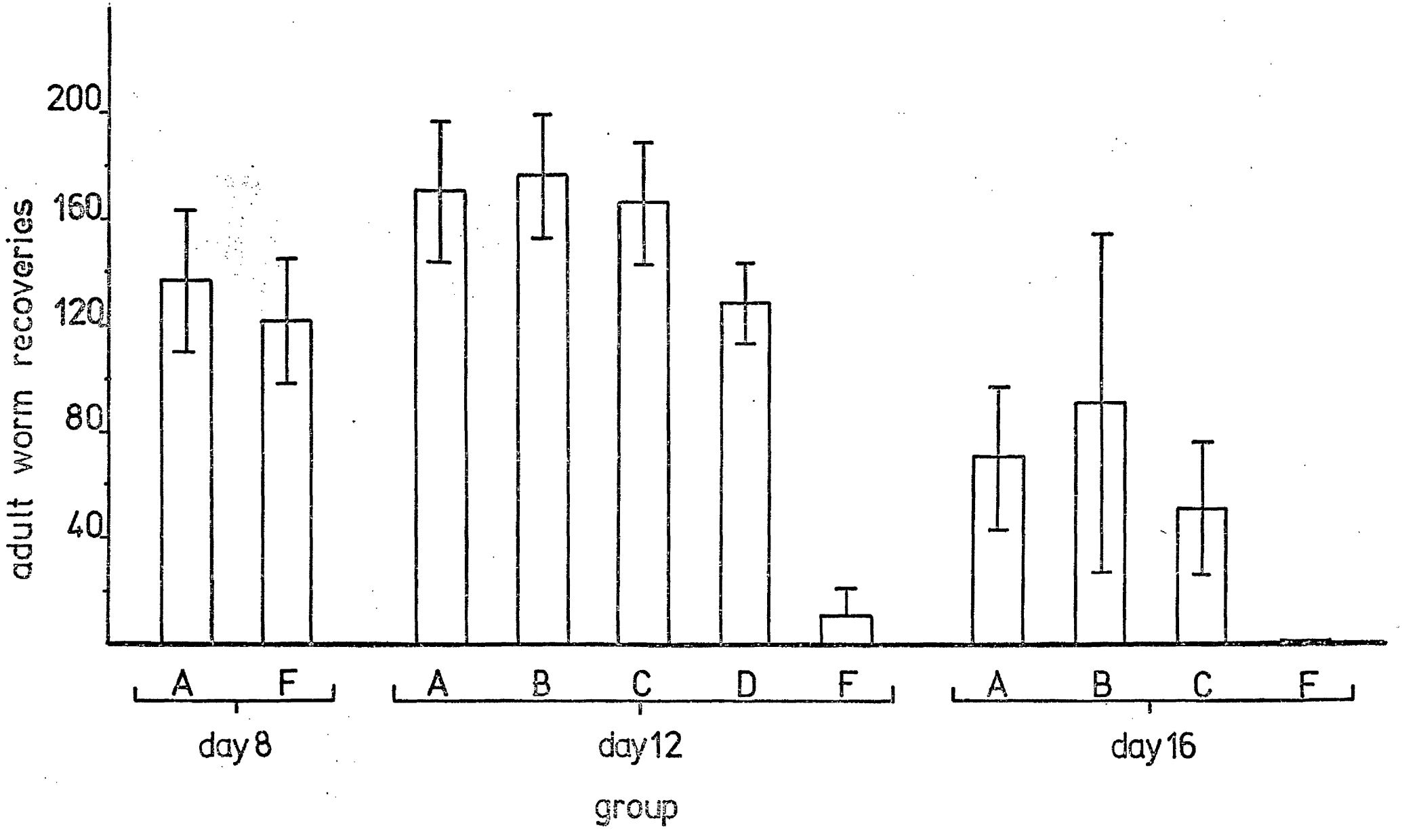
FIGURE 2-34

Effect of Irradiation on the immunosuppressive action of *N. dubius*

Mean recovery (MWR $\pm$ SD) *T. spiralis* at days 8, 12 and 16 post infection

GROUP		
A	300 Nd	+ Tsp
B	300 Nd (7.5 K.rad)	+ Tsp
C	300 Nd (15 K.rad)	+ Tsp
D	1500 Nd (15 K.rad)	+ Tsp
E	300 Nd	
F		Tsp only


adult worm recoveries
200
160
120
80
40
A
F
A
B
C
D
F
A
B
C
F
day 8
day 12
day 16
group

STIMULATION OF IMMUNITY TO NEMATOSPIROIDES DUBIUS IN MICE USING LARVAE ATTENUATED BY COLBALT 60 IRRADIATION

To be published in Parasite Immunology.

SUMMARY

Infective larvae of Nematospiroides dubius attenuated by cobalt 60 irradiation are extremely effective at stimulating immunity. Previously, such levels of protection could only be obtained with multiple immunizations of normal larvae. The critical factor underlying this protective response appears to be the dose of irradiation given to the immunizing infection. Various doses of irradiation have been tested and the most effective of these range between 10 - 30 Krad. The experiments show that provided this level of irradiation is used, the number of immunizing infections is relatively unimportant. Such use of irradiated larvae will be of value in attempting to analyse the immune mechanisms which operate against N. dubius. The possible mechanisms of immunity to N. dubius are discussed.

KEY WORDS: Nematospiroides dubius, nematode, immunity, irradiated larvae.

INTRODUCTION

The nematode, Nematospiroides dubius (Heligmosomoides polygyrus) in mice provides a convenient laboratory model of a chronic parasitic infection. After an 8-day tissue phase in the muscular wall of the gut, the parasites emerge to the lumen of the small intestine, where, as adults, they can survive for up to 8 months (Ehrenford 1954).

Coupled with this long survival is the failure of a primary infection to stimulate protective immunity to a subsequent challenge. Most attempts to stimulate immunity have used multiple immunization schedules involving a series of infections and drug treatments (Behnke and Parish 1979a). As shown by Prowse, Ey and Jenkin (1978), two infections would appear to be the minimum effective immunizing dose. They found one dose to be ineffective but obtained greater than 95% protection with two doses.

The use of irradiation-attenuated parasites to stimulate immunity has never been attempted in this system, although the effects of cobalt 60 irradiation on the survival of the parasite have been reported (Behnke, Parish and Hagan 1980). If irradiation-attenuated parasites proved to be effective it would provide an extremely useful tool with which to evaluate the immune mechanisms that operate, often ineffectively, against N. dubius.

This paper describes the results of experiments designed to determine firstly, whether irradiated parasites do stimulate immunity, and secondly, the combination of dose of irradiation and number of immunizing doses that is most effective in stimulating this immunity.

MATERIALS AND METHODS

Experiments were carried out in both Nottingham and Glasgow Universities.

ANIMALS

Inbred male NIH mice were used in all experiments. These were purchased from Hacking and Churchill Limited (Huntingdon) or bred under conventional animal house conditions in the Zoology Department of Nottingham University. Mice, 6-8 weeks old at the start of each experiment, were killed in groups of six or seven.

NEMATOSPIROIDES DUBIUS

The strain of N. dubius used in the present study was obtained in 1975 from the Wellcome Research Laboratories, Beckenham, and has since been maintained in outbred CFLP mice. The maintenance of the parasite, and the methods used for infection and recovery of worms have already been described (Behnke and Wakelin 1977, Jenkins and Behnke 1977).

IRRADIATION OF INFECTIVE LARVAE

Larvae were irradiated using a cobalt 60 source as described by Behnke et al. (1980).

ANTHELMINTIC

Pyrantel embonate (strongid-P paste, Pfizer) was used to remove adult N. dubius from infected mice. A dose of 100 mg/kg was administered orally as an aqueous suspension. This dose level is known to be adequate for the removal of all adult worms from the intestinal lumen (Behnke and Wakelin 1977).

FAECAL EGG COUNTS

Half a gram to one gram of fresh faeces collected each morning from the pooled faeces of all the mice in each group was dispersed in 30ml of 50% saturated saline. This suspension was washed through a sieve (aperture size 300 microns) with 100% saturated saline and the eggs were counted after flotation in standard McMaster counting slides as described by Gordon and Whitlock (1939).

The counts were expressed as the number of eggs per gram of faeces.

STATISTICAL ANALYSIS OF RESULTS

The results were analysed by the non-parametric Wilcoxon test. A value of $P < 0.05$ was considered to be significant.

RESULTS

EFFECT OF IMMUNIZATION WITH NORMAL OR IRRADIATED LARVAE

In the first experiment (Experiment 53), two groups of mice were infected with 200 normal or 200, 10 Krad irradiated larvae on day 0 and both these groups, plus a group of age matched controls, were treated with pyrantel on days 34 and 35. All the groups were infected with 100 normal larvae on day 42 and mice were killed on 14 and 35 days post challenge. The results are shown in Figure 2-35.

A second experiment (Experiment 54) using a shorter immunizing period and 25 Krad irradiated larvae was carried out. Two groups of mice were infected with 100 normal or 100 irradiated larvae on day 0 followed by pyrantel treatment on days 14 and 15. Challenge infection was given on day 28 and all groups, including challenge controls, were killed on day 14 post challenge. The results are shown in Table 7.

In Experiment 53, larvae irradiated at 10 krad clearly conferred greater protection against a subsequent challenge than did normal larvae, as assessed by worm recoveries on both day 14 and day 35 post challenge. Although, in terms of numbers of worms recovered, there was no significant difference in immunizing capacity between normal and 25 Krad irradiated larvae in Experiment 54, unlike Experiment 53, the challenge worms recovered were smaller and less mature than those recovered from mice immunized with normal larvae. It would seem, therefore, that mice immunized with irradiated larvae are more capable of arresting the development (see Behnke and Parish 1979b) of a subsequent challenge than are mice immunized with normal larvae.

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FIGURE 2-35

Mean worm recoveries, days 14 and 35 post challenge.

Mice immunized with 200  normal larvae, or  (10 K.rad) irradiated larvae. Control group,  no previous infection. Challenge infection 100 normal N. dubius larvae.

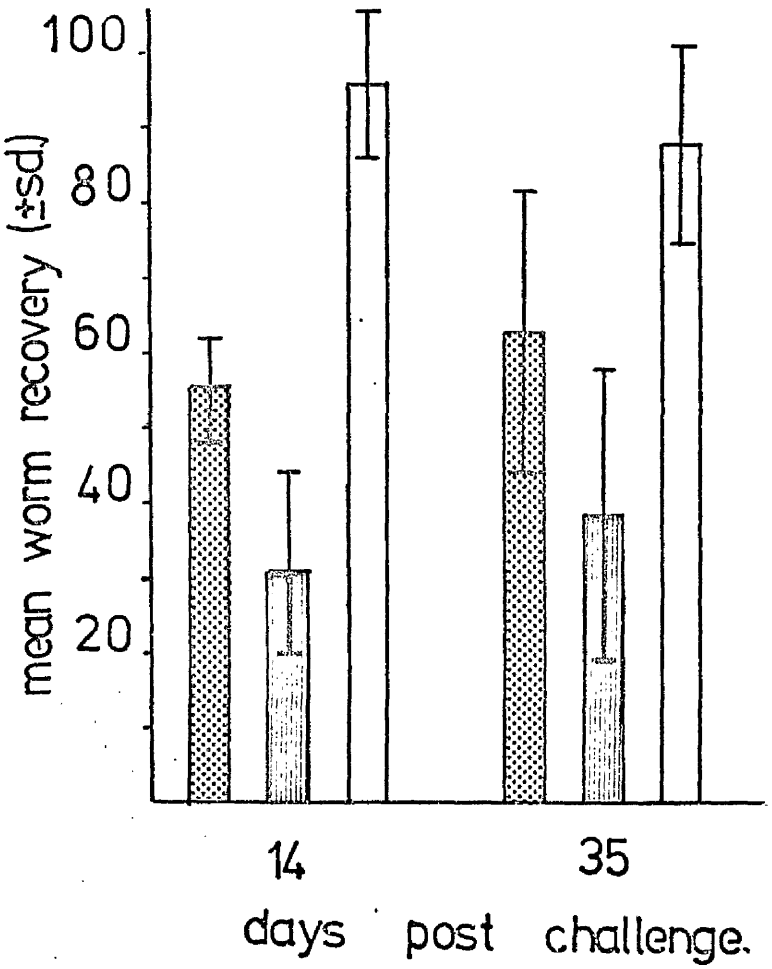
100
80
60
40
20
mean worm recovery (±sd)
14
35
days post challenge.

TABLE 7

Experiment 54.

Comparison of normal and irradiated larvae in stimulation of immunity

	Immunization	Worm recoveries (Mean ± S.D.) Day +14 post challenge (100 *N. dubius*)
A	1 x 100 (25 Krad) larvae	51 ± 14.5
B	1 x 100 (normal) larvae	72 ± 10.5
C	No immunization	92 ± 10.1

A vs C, B vs C, $P < 0.05$ both combinations

EFFECT OF DIFFERENT DOSES OF IRRADIATION ON IMMUNOGENICITY OF N. DUBIUS LARVAE

The above results showed that irradiated larvae were more effective at stimulating immunity than normal larvae. An investigation of the effect of varying the dose of irradiation on the immunogenicity of larvae was undertaken. A total of five experiments was carried out. In two of these, a protocol similar to that of Experiment 53 was used, but with mice killed on day 35 post challenge only and mice irradiated at 5, 10, 15, 20, 30 and 40 Krad. The combined results are shown in Figure 2-36 (Experiment 55). In a further experiment groups of mice were infected with 300 normal, 7.5, 15 or 25 Krad irradiated larvae, treated with pyrantel on days 14 and 15, and challenged with 100 normal larvae on day 18. All the mice were killed 14 days later. The results are shown in Table 8 (Experiment 56).

In both these experiments, the protective effect of immunizing with irradiated larvae increased with increasing dose of irradiation. Although this is evident from the mean worm recoveries shown in Figure 2-36, the variation within the groups was large. However, as the figures shown above the barlines indicate, the numbers of mice in each group which were >90% protected against a challenge infection increased with increasing dose of irradiation.

EFFECT OF VARYING THE NUMBER OF IMMUNIZING DOSES ON THE RESPONSE TO CHALLENGE INFECTION

In this experiment (Experiment 57) all immunizing doses were of 300 normal or 300, 25 Krad irradiated larvae. Four groups of mice were immunized at 16 day intervals with 4x, 3x, 2x and 1x 300, 25 Krad irradiated larvae and two other groups were immunized with 4x and 1x normal larvae. Each immunizing dose was terminated using pyrantel

TABLE 8

Experiment 56.

Effect of dose of irradiation on immunity stimulated by *N. dubius* larvae

	Immunization	Worm recoveries (Mean ± S.D.) Day +14 post challenge (100 *N. dubius*)
A	1 x 300 (25 Krad) larvae	58 ± 9.6
B	1 x 300 (15 Krad) larvae	71 ± 10.6
C	1 x 300 (7.5 Krad) larvae	77 ± 9.9
D	1 x 300 (normal) larvae	85 ± 10.6
E	No immunization	91 ± 11.0

A vs C, A vs D, A vs E, B vs D, B vs E, $P < 0.05$ all combinations.

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FIGURE 2-36

Mean worm recoveries, day 35 post challenge from mice immunized with 300 larvae irradiated at 0, 5, 10, 15, 20, 30 and 40 K.rad. Control group, no previous infection  . Challenge infection 100 normal N. dubius larvae. Figures above barlines indicate the number of mice in each group, 90% protected, against challenge.

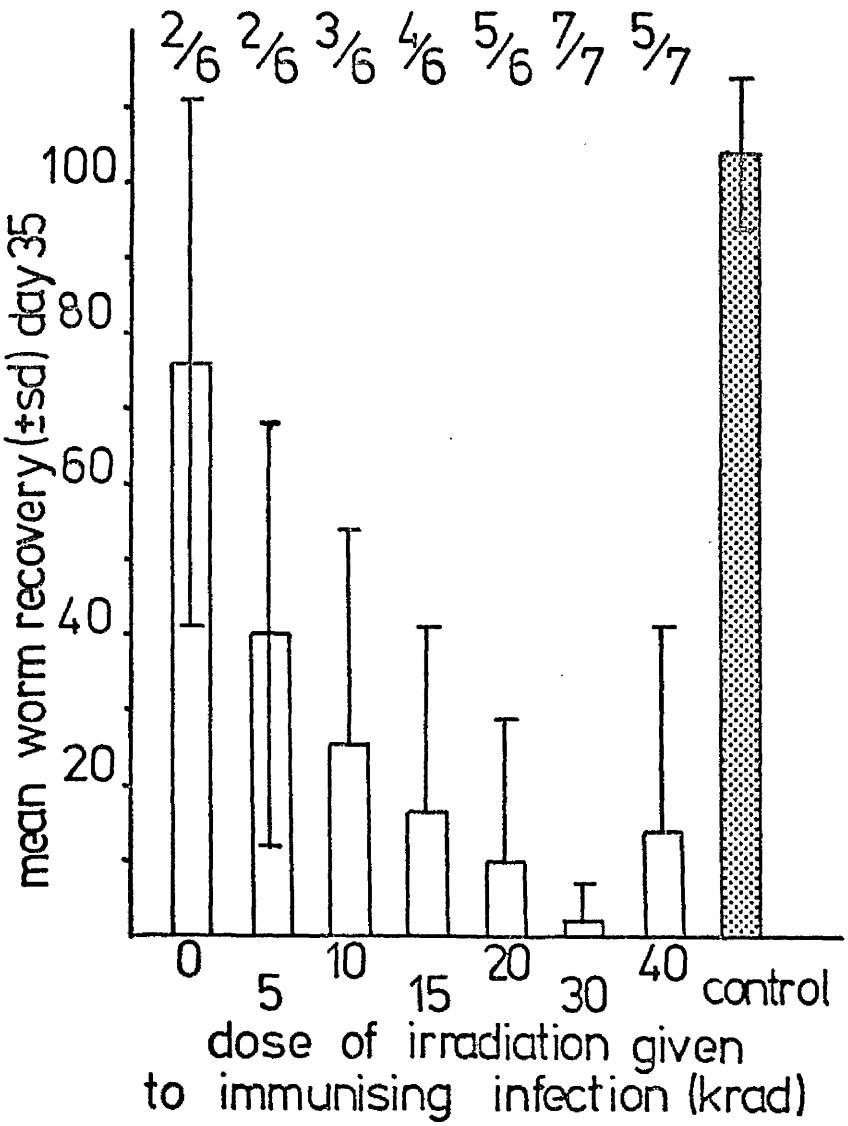
2/6
2/6
3/6
4/6
5/6
7/7
5/7
100
80
60
40
20
mean worm recovery (±sd) day 35
0
5
10
15
20
30
40
control
dose of irradiation given
to immunising infection (krad)

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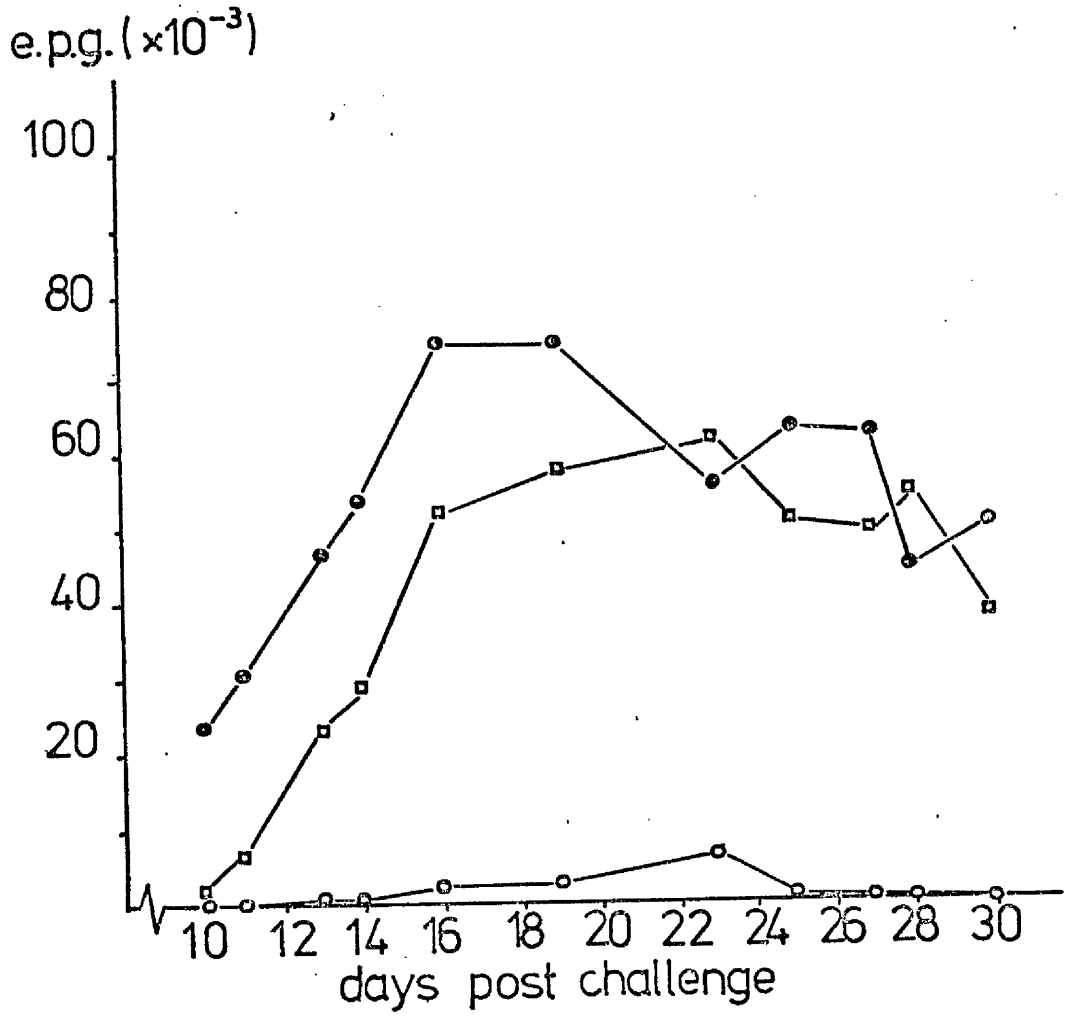
FIGURE 2-37

Pattern of egg production in groups of mice after challenge infection with 100 normal *N. dubius* larvae.

- □ immunized 1 x 300 normal *N. dubius*
- O immunized 1 x 300 (25 K.rad) *N. dubius*, prior to challenge.
- ● Control group, no previous infection.

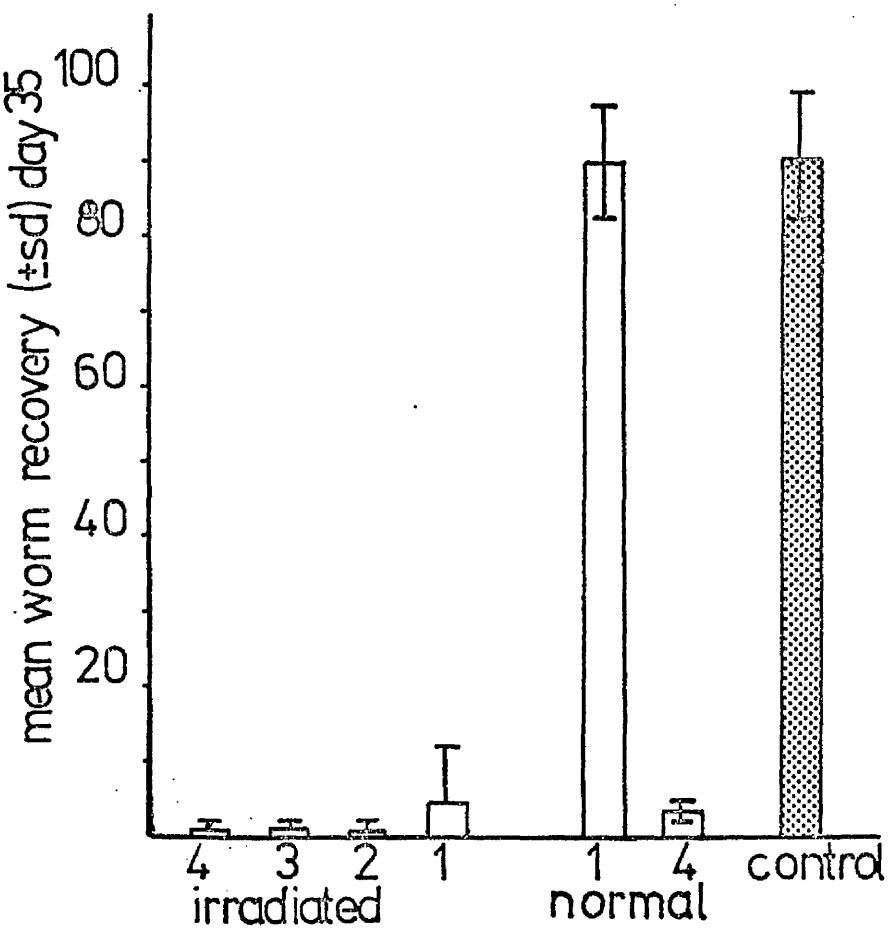
Counts expressed as eggs per gram of faeces.


e.p.g. (×10⁻³)
100
80
60
40
20
10 12 14 16 18 20 22 24 26 28 30
days post challenge

Facing page 200

FIGURE 2-38

Mean worm recoveries, day 35 post challenge with 100 normal *N. dubius* larvae. Groups of mice immunized 1x, 2x, 3x and 4x 300 (25 K.rad) irradiated *N. dubius* or 1x and 4x normal *N. dubius* prior to challenge. Control group  no previous infection.


mean worm recovery (±sd) day 35
100
80
60
40
20
4
3
2
1
1
4
control
irradiated
normal

after 14 days. All groups, including age matched controls were challenged with 100 normal larvae and killed on day 35 post challenge. Faecal egg counts from these groups are shown in Figure 2-37 and day 35 post challenge worm recoveries in Figure 2-38. This experiment again showed a clear difference between the effectiveness of normal and irradiated larvae. As assessed by day 35 worm recoveries, immunization with 1x 300, 25 krad larvae provided more than 95% protection against challenge, whereas 1x 300 normal conferred no protection. However, immunization with the latter did delay the onset of patency but the egg counts reached control levels at day 16 whereas egg counts from mice immunized with 1x 300, 25 Krad larvae, even at maximum levels, reached only one tenth of control output. On no occasion were eggs recorded from mice immunized with 4x normal larvae or mice immunized with 2x, 3x and 4x, 25 Krad irradiated larvae.

DISCUSSION

As Mulligan et al (1961) have shown, successful use of irradiated larvae as immunizing agents against nematode infections depends upon the selection of the most effective components from a large number of variables. In the mouse-N. dubius system we have shown quite conclusively that irradiated N. dubius larvae are effective at stimulating immunity against a subsequent challenge and that the most important variable is the level of irradiation in attenuation. The number of immunizing doses given is relatively unimportant provided the correct dose of irradiation has been used. We have further evidence (unpublished) to suggest that the size of the immunizing inoculum is not an important variable.

Although the exact details of survival and development of irradiated larvae are not known, the levels of irradiation used in our

experiments prevent normal maturation and emergence of the larvae. As a result, the host is exposed for a prolonged period to larval antigens and it is this we believe that stimulates high levels of protective immunity. This view is supported by the fact that single infections of normal larvae fail to stimulate protective immunity, whereas multiple infections which extend the duration of host exposure to larval stages, can produce strong immunity (see Experiment 57). Further evidence can be drawn from the fact that the least effective stimulation of immunity by irradiated larvae was observed with those irradiated at 5 Krad; these are known to develop and emerge into the lumen at the same time as normal larvae (Behnke et al. 1980).

Prowse et al. (1979) have shown that different mouse strains subjected to similar immunizing schedules develop different levels of resistance to a subsequent challenge infection. The high level of protection, 95% obtained here using one immunization with 300 (25 Krad) cobalt 60 irradiated larvae, is no doubt due in part to the fact that these experiments have been conducted in NIH mice which are known to respond rapidly to nematode infections (Wakelin 1980). Whether or not all mouse strains respond as well as do NIH to immunization with irradiated larvae is the subject of a further investigation. The steady rise in the number of responding mice in the groups of Experiment 55, a phenomenon which is more characteristic of outbred rather than inbred strains of mice, suggests that even for NIH mice there is a threshold level of antigenic stimulation which must be exceeded before an effective response can be mounted.

The host mechanisms which act against N. dubius are not well understood. Jones and Rubin (1974) have shown the presence of macrophages and eosinophils in the granulomatous lesions which develop

around larval stages in the intestinal wall; eosinophils were associated particularly with larval dissolution. Chaicumpa and Jenkin (1978) and Prowse et al. (1978) have suggested that macrophages are responsible for the partial immunity resulting from a single immunizing infection; macrophages from these mice have been shown to adhere to infective third stage larvae in vivo. However, only after two immunizing infections with normal larvae did they obtain more than 95% protection. Coinciding with this protection was a blood eosinophilia and the appearance of eosinophils in peritoneal exudates. They concluded that eosinophils may be involved in a second immune mechanism which gives rise to this high level of protection. As yet we have not determined whether eosinophils appear after a single immunization with cobalt 60 irradiated larvae.

By attenuating larvae with cobalt 60 irradiation, the characteristics of infection with N. dubius have been dramatically altered. Single infections with normal larvae produce only poor immunity which is manifested by a delay in the onset of patency, possibly due to a slight delay in larval development. Irradiated larvae, on the other hand, produce a highly protective immunity which greatly reduces the number of parasites reaching maturity. The results of this work indicate that once activated, the immune mechanisms of NIH mice, which normally fail to work against a single N. dubius infection, are extremely effective. Irradiated larvae will provide a means by which the underlying mechanisms of immune responsiveness and non-responsiveness in various strains of mice can be studied.

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REFERENCES

BEHNKE, J.M. & PARISH, H.A. (1979a) Expulsion of Nematospiroides dubius from the intestine of mice treated with immune serum. Parasite Immunology 1, 13.

BEHNKE, J.M. & PARISH, H.A. (1979b) Nematospiroides dubius: arrested development of larvae in immune mice. Experimental Parasitology 47, 116.

BEHNKE, J.M., PARISH, H.A. & HAGAN, P. (1980) The effect of gamma irradiation on Nematospiroides dubius. Factors affecting the survival of worms in a primary infection in mice. Journal of Helminthology (in press).

BEHNKE, J.M. & WAKELIN, D. (1977) Nematospiroides dubius: stimulation of acquired immunity in inbred strains of mice. Journal of Helminthology 51, 167.

CHAICUMPA, V. & JENKIN, C.R. (1978) Studies *in vitro* on the reaction of peritoneal exudate cells from mice immune to infection with *Nematospiroides dubius* with the infective third stage larvae of this parasite. *Australian Journal of Experimental Biology and Medical Science* 56, 61.

EHRENFORD, F.A. (1954) The life cycle of *Nematospiroides dubius* Baylis (Nematoda: Heligmosomidae). *Journal of Parasitology* 40, 480.

GORDON, H.M. & WHITLOCK, H.V. (1939) A new technique for counting nematode eggs in sheep faeces. *Journal of the Council for Scientific and Industrial Research, Australia* 12, 50.

JENKINS, S.N. & BEHNKE, J.M. (1977) Impairment of primary expulsion of *Trichuris muris* in mice concurrently infected with *Nematospiroides dubius*. *Parasitology* 75, 71.

JONES, C.E. & RUBIN, R. (1974) *Nematospiroides dubius*: Mechanisms of host immunity I. Parasite counts, histopathology and serum transfer involving orally or subcutaneously sensitised mice. *Experimental Parasitology* 35, 434.

MULLIGAN, W., GORDON, H.McL., STEWART, D.F. & WAGLAND, B.M. (1961) The use of irradiated larvae as immunizing agents in *Haemonchus contortus* and *Trichostrongylus colubriformis* infections of sheep.

PROWSE, S.J., EY, P.L. & JENKIN, C.R. (1978) Immunity to *Nematospiroides dubius*: cell and immunoglobulin changes associated with the onset of immunity in mice. *Australian Journal of Experimental Biology and Medical Science* 56, 237.

PROWSE, S.J., MITCHELL, G.F., EY, P.L. & JENKIN, C.R. (1979) The development of resistance in different inbred strains of mice to infection with Nematospiroides dubius. Parasite Immunology 1, 277.

WAKELIN, D. (1980) Genetic control of immunity to parasites. Infection with Trichinella spiralis in inbred and congenic mice showing rapid and slow responses to infection. Parasite Immunology (in press).

EFFECT OF SIZE OF IMMUNISING DOSE ON RESPONSE TO CHALLENGE INFECTION

In this experiment (Experiment 58) larvae were irradiated at 25 K.rad and groups of mice were infected at different dose levels. All groups, except controls, were treated with pyrantel on days 14 and 16, and all seven groups were infected with 100 N. dubius (normal larvae) on day +18. Groups of six mice (male NIH) were killed 14 days post challenge. The experimental design is shown below and the worm recoveries in Figure 2-39.

GROUP	DAY				
	0	14	16	18	32
A	50 Nd ↯	PYR	PYR	100 Nd	K
B	100 Nd ↯	PYR	PYR	100 Nd	K
C	200 Nd ↯	PYR	PYR	100 Nd	K
D	300 Nd ↯	PYR	PYR	100 Nd	K
E	50 Nd Control	PYR	PYR	100 Nd	K
F	300 Nd Control	PYR	PYR	100 Nd	K
G	-	-	-	100 Nd	K

Group G gave the expected recovery from an infection with 100 normal larvae. Groups E and F immunised with 50 and 300 N. dubius respectively gave only a slight reduction in mean worm recovery. A more marked effect was shown in the groups immunised with 100, 200 and 300, 25 K.rad irradiated N. dubius. These three groups (Groups B, C and D) showed a similar reduction in challenge infection mean worm recovery; in all the recoveries were statistically significantly reduced compared with controls but were not significantly different from one another. Group A mice, immunised with only 50 irradiated N. dubius showed a 20-25% reduction on control mean worm recovery (Group G).

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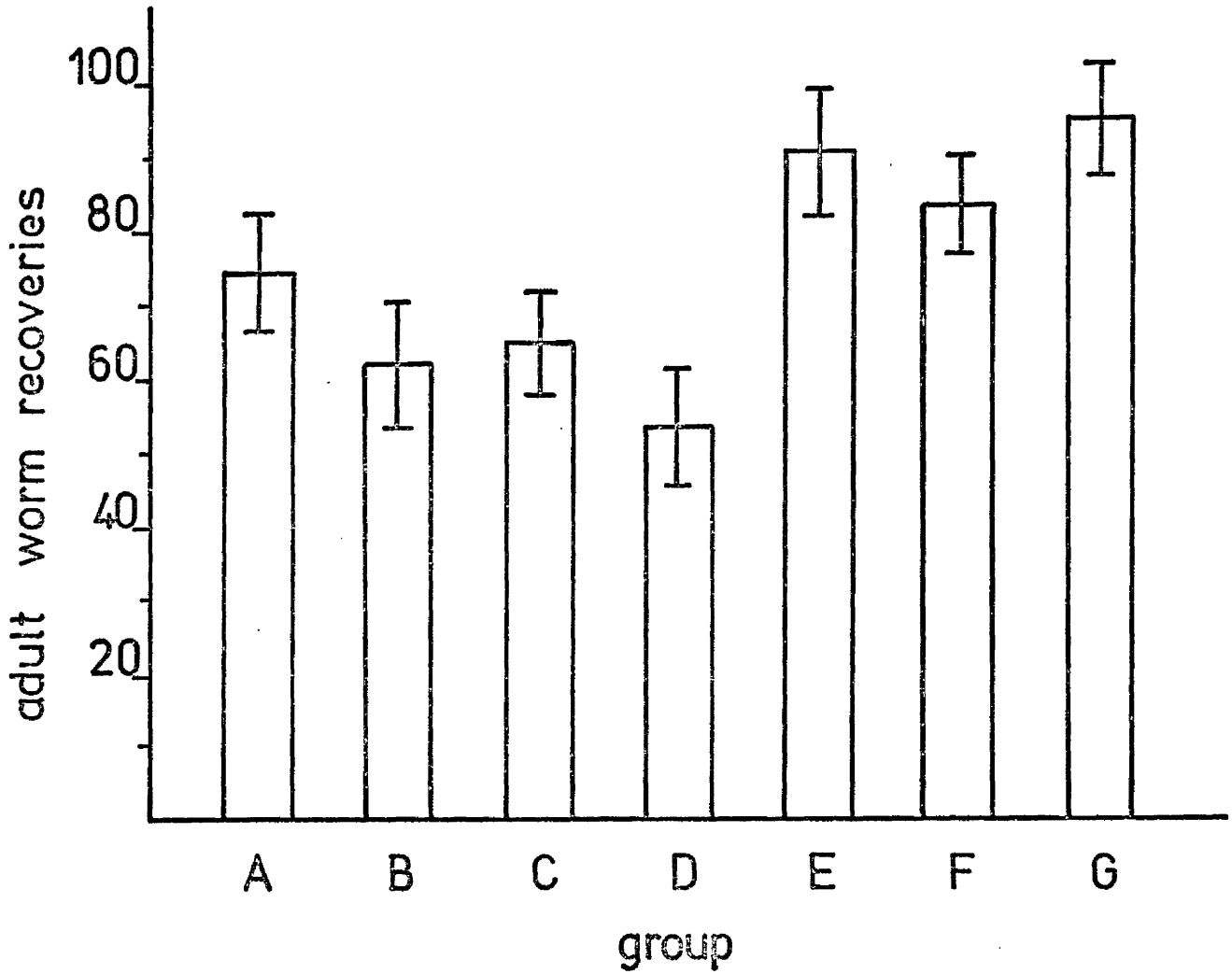
FIGURE 2-39

Effect of size of immunizing dose of 25 K.rad irradiated larvae on response to challenge infection with 100 normal *N. dubius* larvae

GROUP	IMMUNIZING INFECTION	CHALLENGE INFECTION
A	50 Nd ↯	100 Nd normal larvae
B	100 Nd ↯	100 Nd normal larvae
C	200 Nd ↯	100 Nd normal larvae
D	300 Nd ↯	100 Nd normal larvae
E	50 Nd normal	100 Nd normal larvae
F	300 Nd normal	100 Nd normal larvae
G	-	100 Nd normal larvae

Mean recovery (MWR $\pm$ SD) *N. dubius* 14 days post challenge


adult worm recoveries
100
80
60
40
20
A
B
C
D
E
F
G
group

These results suggest that, down to a lower limit of 100 N. dubius, the size of the immunising infection is not important in determining the resulting immunity to challenge infection.

EFFECT OF DELAYING CHALLENGE FOLLOWING IMMUNISATION WITH 25 K.RAD IRRADIATED LARVAE

This experiment (Experiment 59) was designed to determine whether or not the immunity induced by irradiated N. dubius larvae was long lasting. Also, with the delayed challenge infection the response initiated by the immunising infection will have had time to subside whereas with earlier experiments the challenge infection larvae may have been caught up in the response against the first (immunising) infection. The experimental design is shown below:

GROUP	DAY			
	0	14	28	45
A	Nd⚡	Nd⚡	Nd⚡	100 Nd
B	Nd⚡	-	-	100 Nd
C	-	-	Nd⚡	100 Nd
D	Nd	Nd	Nd	100 Nd
E	Nd	-	-	100 Nd
F	-	-	Nd	100 Nd
G	-	-	-	100 Nd

All immunising infections were of 300 larvae and all immunised groups were treated with pyrantel 12 and 14 days after each immunising infection. Egg counts for all groups were recorded for the first 30 days of the challenge infection. All groups were killed on day 35 post challenge and the mean worm recoveries are shown in Figure 2-40, egg counts in Figure 2-41.

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FIGURE 2-40

Effect of delaying challenge following immunization with 25 K.rad irradiated larvae

Mean recovery (MWR $\pm$ SD) N. dubius day 35 post infection with 100 N. dubius

GROUP	DAY			
	0	14	28	45
A	Nd⚡	Nd⚡	Nd⚡	100 Nd
B	Nd⚡	-	-	100 Nd
C	-	-	Nd⚡	100 Nd
D	Nd	Nd	Nd	100 Nd
E	Nd	-	-	100 Nd
F	-	-	Nd	100 Nd
G	-	-	-	100 Nd

All immunizing infections were of 300, 25 K.rad (Nd⚡) irradiated larvae or 300 normal larvae (Nd).

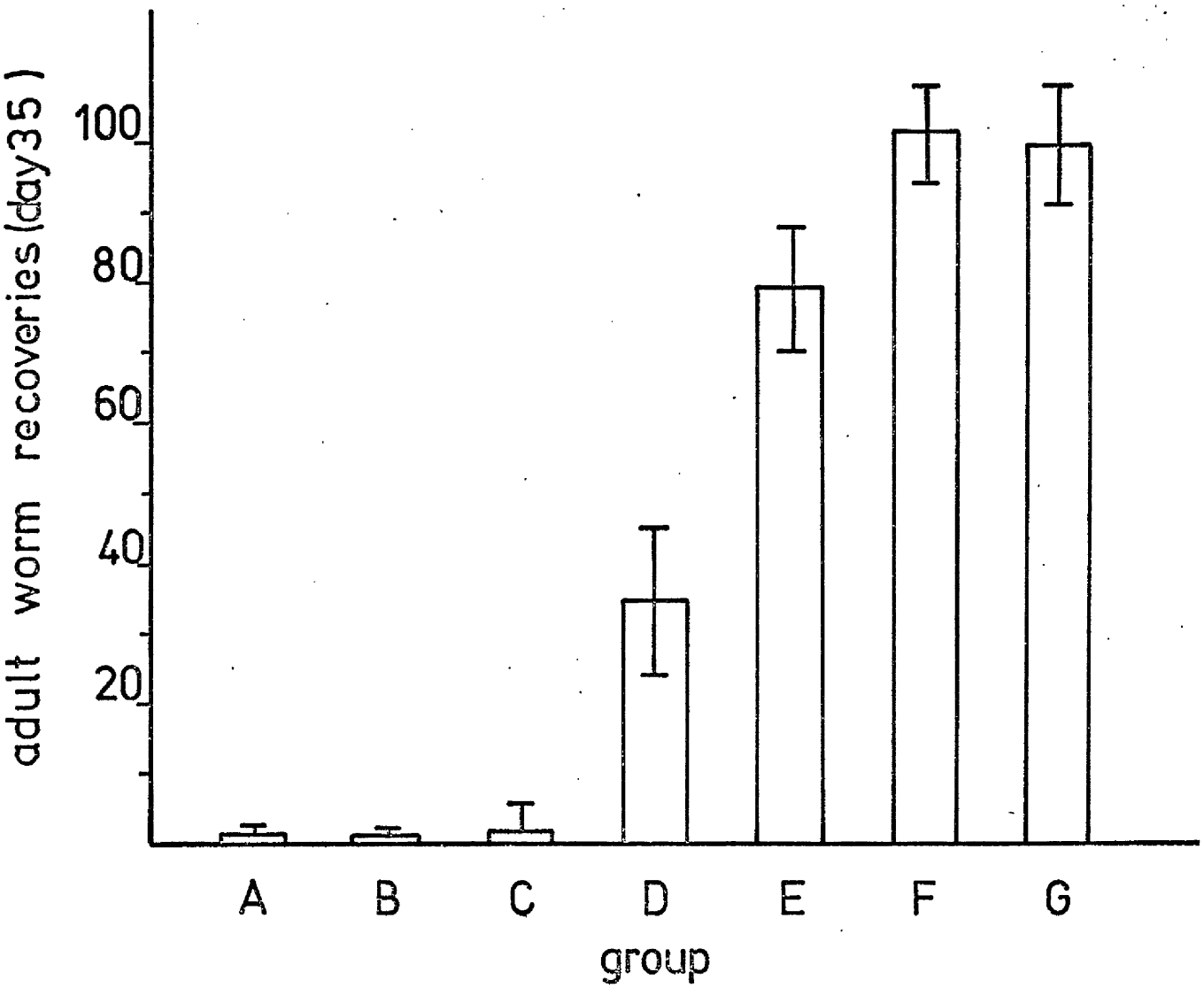
adult worm recoveries (day 35)
100
80
60
40
20
A
B
C
D
E
F
G
group

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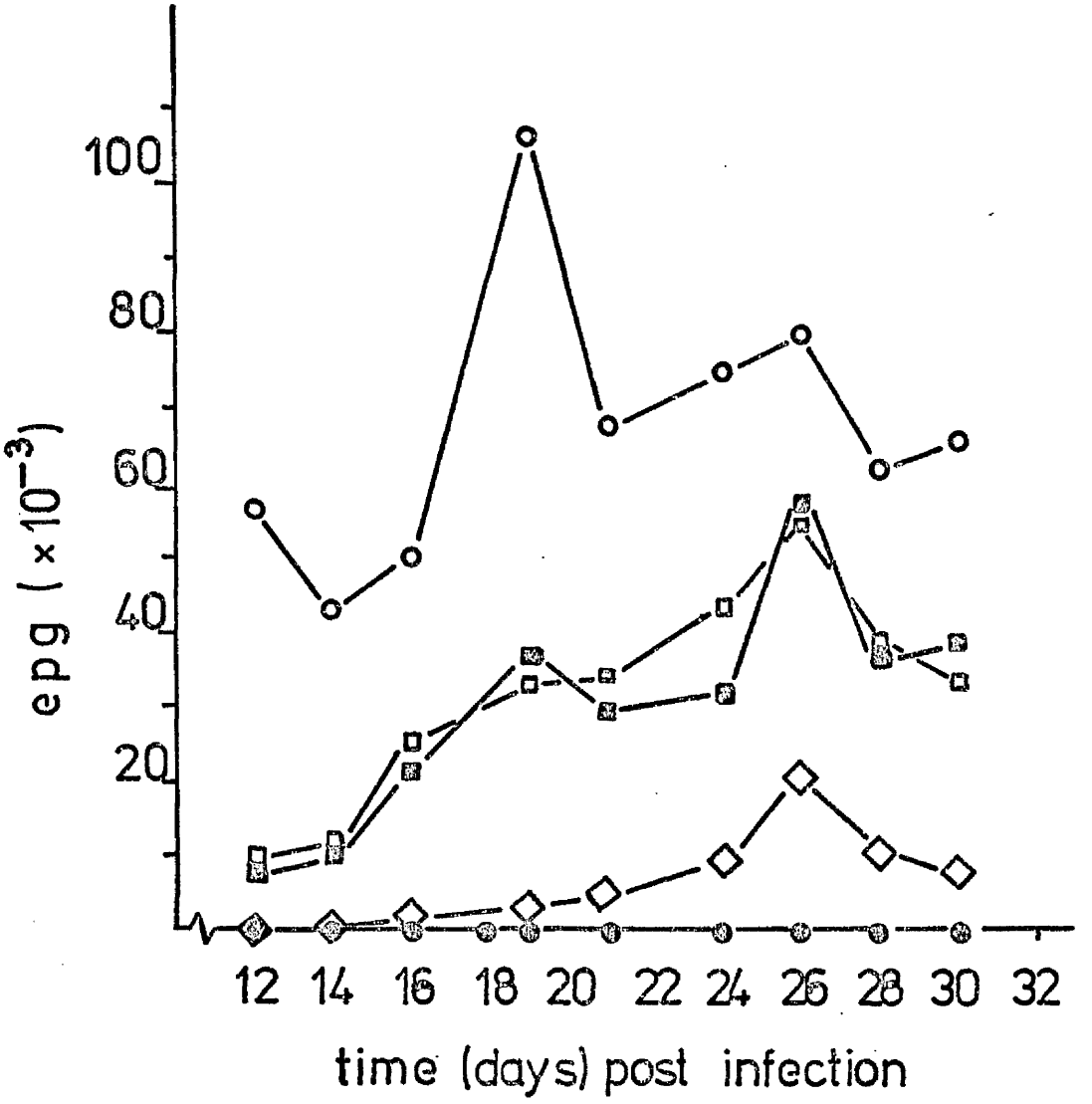
FIGURE 2-41

Effect of delaying challenge following immunization with 25 K.rad irradiated larvae

Egg counts (e.p.g. x 10^{-3}) from groups shown in Figure 2-40

A	●	)
B	●	) All immunized irradiated larvae
C	●	)
D	◇	)
E	■	) All immunized normal larvae
F	□	)
G	○	Control


epg ($\times 10^{-3}$)
100
80
60
40
20
12 14 16 18 20 22 24 26 28 30 32
time (days) post infection

All groups immunised with irradiated larvae (Groups A, B and C) had a mean worm recovery 95% lower than that of the control Group G. This suggests that the time between immunisation with irradiated larvae and subsequent challenge was unimportant in determining the level of response to the challenge (Group B, delayed challenge, Group C early challenge). Also one dose of irradiated larvae (Groups B and C) was as effective as 3 doses (Group A) in the level of immunity to challenge produced. This was not the case with the groups immunised with normal larvae. Mice immunised with 3 infections of normal larvae (Group D) were 65% protected against challenge infection whereas Group E (1 immunising dose, delayed challenge) mice were only 20% protected. The mice immunised with one dose of normal larvae and challenged soon afterwards (Group F) were not protected against challenge infection when compared with control (Group G). No eggs were found in the faeces of mice immunised with irradiated larvae (Groups A, B and C, Figure 2-41). The groups immunised with 300 normal larvae (Groups E and F) gave similar egg counts which were higher than those of mice (Group D) immunised with 3 x 300 normal N. dubius larvae. All these groups gave egg counts lower than those of the control group, Group G.

DOES IMMUNISATION WITH IRRADIATED LARVAE (25 K.rad) PRODUCE AN IMMUNITY WHICH CAN BE TRANSFERRED WITH MLNC?

Previous attempts at transfer had been made with MLN taken from donors 'immunised' by infection(s) with normal larvae. In this experiment (Experiment 60) MLNC were taken from mice immunised with irradiated larvae and normal larvae. The immunising schedule is shown below:

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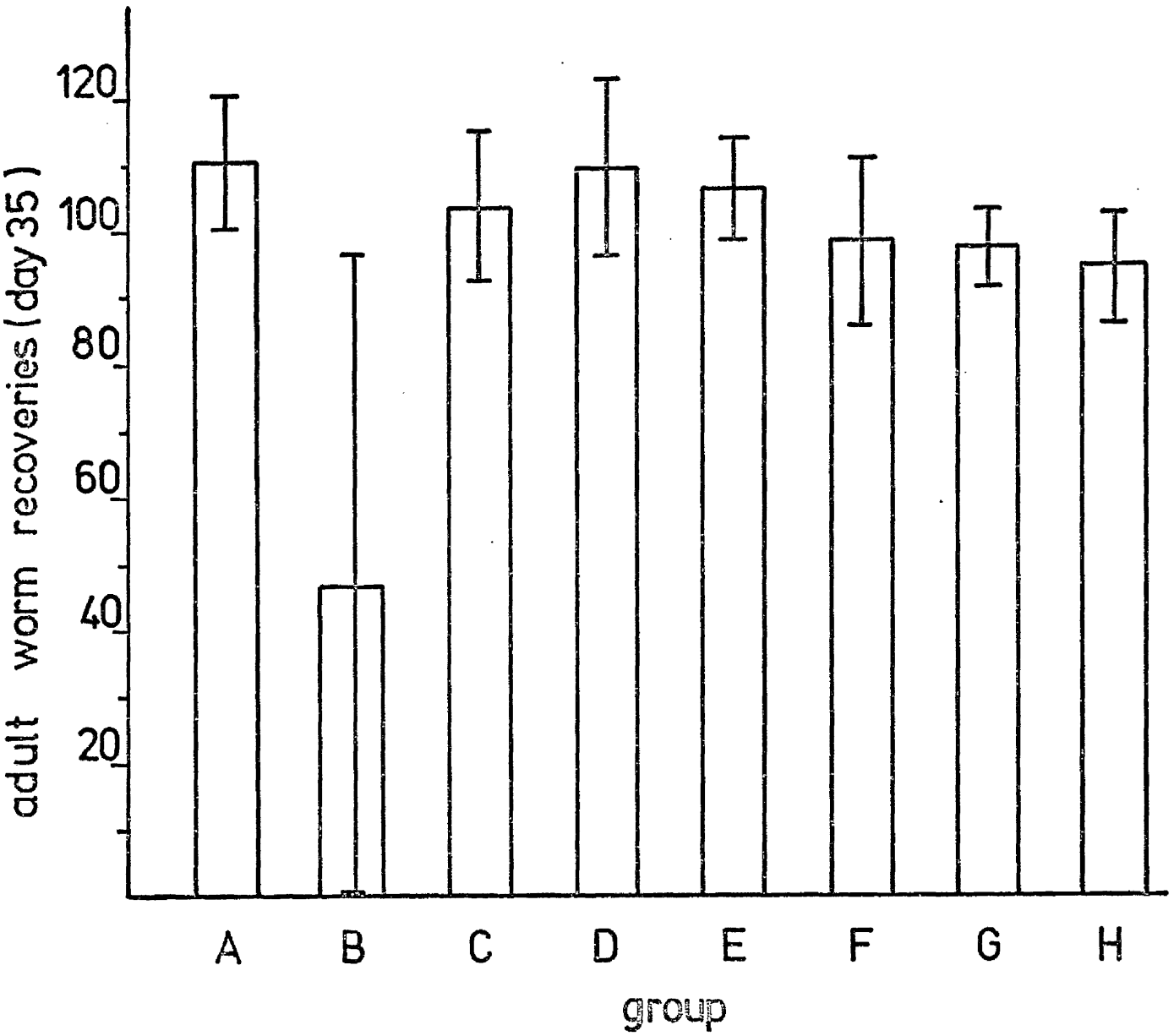
FIGURE 2-42

Effect of transfer of MLNC from mice immunized with irradiated and control larvae on the response to *N. dubius* infection

Mean recovery (MWR ± SD) *N. dubius* day 35 post infection with 100 *N. dubius*

GROUP	DONORS		
A	4 x 300 (Nd ϟ)	+ 100 Nd 3 x 10^7 MLNC (day 35)	100 Nd
B	3 x 300 (Nd ϟ)	+ 100 Nd 3 x 10^7 MLNC (day 35)	100 Nd
C	2 x 300 (Nd ϟ)	+ 100 Nd 3 x 10^7 MLNC (day 35)	100 Nd
D	1 x 300 (Nd ϟ)	+ 100 Nd 3 x 10^7 MLNC (day 35)	100 Nd
E	4 x 300 (normal Nd)	+ 100 Nd 3 x 10^7 MLNC (day 35)	100 Nd
F	1 x 300 (normal Nd)	+ 100 Nd 3 x 10^7 MLNC (day 35)	100 Nd
G	-	+ 100 Nd 3 x 10^7 MLNC (day 35)	100 Nd
H	-	- no cells, control	100 Nd


adult worm recoveries (day 35)
120
100
80
60
40
20
A
B
C
D
E
F
G
H
group

Facing page 214

FIGURE 2-43

Effect of transfer of MLNC from mice immunized with irradiated and control larvae on the response to *N. dubius* infection

Egg counts (e.p.g. x 10^{-3})

Only 3 groups illustrated

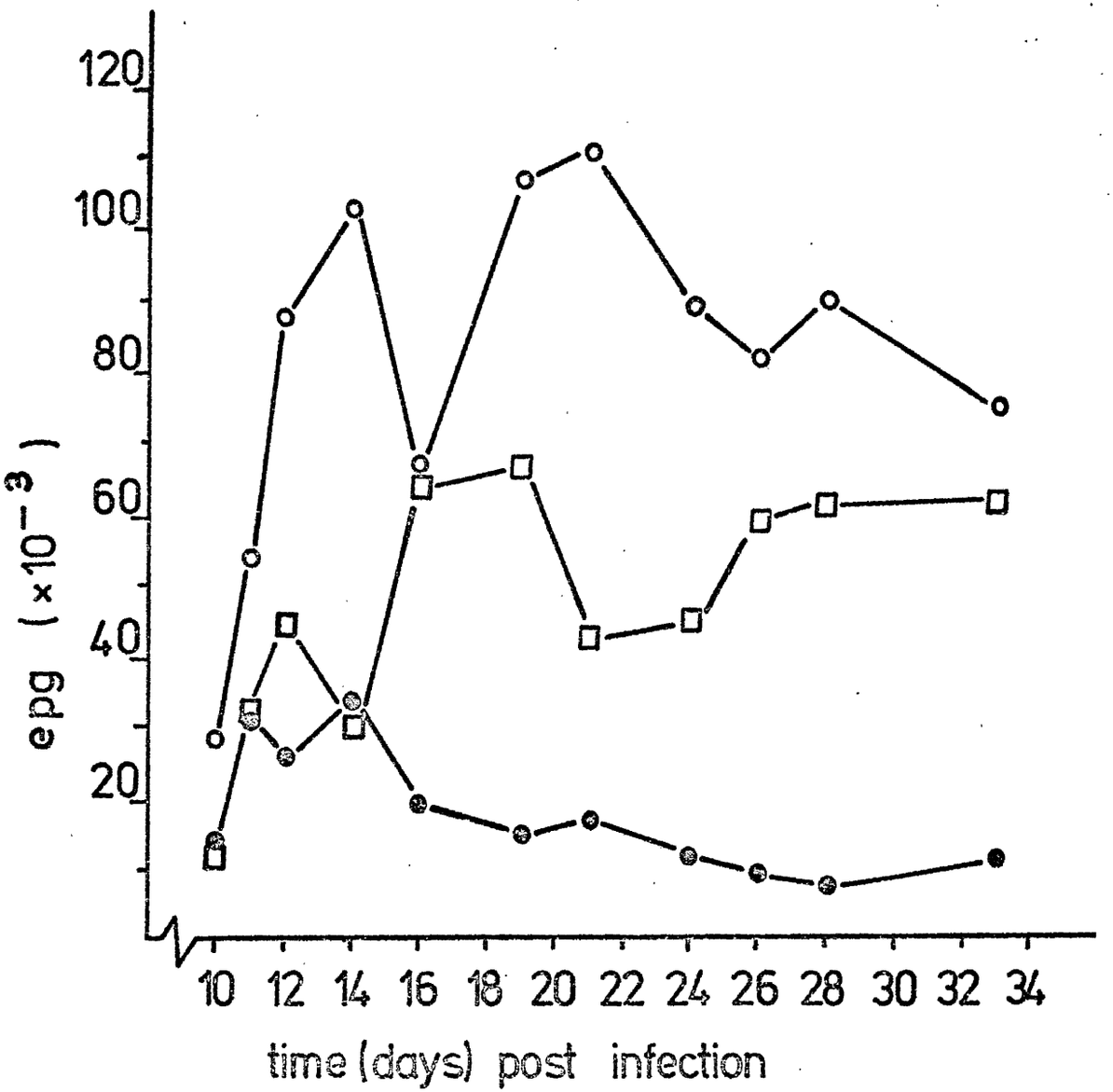
GROUP

B ●

E □

F ○

See Figure 2-42 for details of immunization and MLNC transfer


120
100
80
60
40
20
epg (×10⁻³)
10 12 14 16 18 20 22 24 26 28 30 32 34
time (days) post infection

GROUP	DONORS			RECIPIENTS
A	4 x 300	+ 100 Nd	3×10^7 MLNC (day 35)	100 Nd
B	3 x 300	+ 100 Nd		100 Nd
C	2 x 300	+ 100 Nd		100 Nd
D	1 x 300	+ 100 Nd		100 Nd
E	4 x 300	+ 100 Nd		100 Nd
F	1 x 300	+ 100 Nd		100 Nd
G	-	+ 100 Nd		100 Nd
H	-	+ 100 Nd	no cells, control	100 Nd

Fuller details of the immunising schedule are given on page . The recipients were all infected with 100 N. dubius and egg counts were followed for the first 33 days of the infection. Groups of mice were killed 35 days after transfer/infection and mean worm recoveries are shown in Figure 2-42, egg counts in Figure 2-43. All groups gave mean worm recoveries (day 35) which were similar to those of controls except Group B which had received MLNC from 3 x 300 + 100 N. dubius immunised mice (Figure 2-42). This was the only group which had a significantly decreased egg production throughout the whole experiment (Figure 2-43). Three of the mice in Group B gave worm recoveries of less than 20 whereas the other three gave recoveries greater than 80. This suggests that in three mice at least the MLNC from mice immunised with irradiated larvae had been effective after transfer to recipients.

IMMUNISATION WITH 25 K.RAD IRRADIATED N. DUBIUS IN STRAINS OF MICE

All the immunisation experiments which have been reported here have been done in NIH mice. NIH mice are known to be particularly good responders to helminth parasites so the high levels of protection obtained by using irradiated larvae may be due in part to the responsiveness of the mouse strain used. To answer the question whether the

protection obtained from using irradiated larvae is or is not strain dependent an experiment (Experiment 61) using 4 strains NIH (males), C_{57} black (females), CFLP (females) and F1 generation C_{57} black/NIH (males) was done. Mice were immunised with 2 x 300 (25 K.rad) N. dubius or 2 x 300 N. dubius normal larvae and on days 12 and 14 of each immunising infection had their worm burdens removed using pyrantel. Twenty eight days after the start of the immunising schedule all groups of control mice were challenged with 100 N. dubius and egg counts were followed for the first 29 days of infection. All groups were killed on day 35 post challenge. The mean worm recoveries are shown in Figure 2-44 and egg counts in Figure 2-45. No eggs were ever recovered from any of the strains immunised with two doses of irradiated larvae. The most interesting result was obtained from the C_{57} black mice which were immunised with 2 x 300 normal N. dubius larvae. These mice gave egg counts which were similar to those obtained from the primary infection (challenge control) in C_{57} black mice whereas all the other strains gave greatly reduced egg counts well below control levels. The F1 C_{57} /NIH mice responded like NIH mice not like C_{57} black. The worm recoveries show that all groups immunised with 2 x 300 (25 K.rad) irradiated larvae were well protected against the challenge infection with normal larvae. All groups immunised with 2 x 300 normal N. dubius larvae were also well protected, except the C_{57} black mice. The protection in these groups was never as great as in the groups immunised with irradiated larvae but the protection in the C_{57} black mice (Group G) was particularly poor, although the significance of this result is obscured by the large variation. Observations made at autopsy on the condition of the intestines of mice in the groups are included on Figure 2-44. The interesting point from this is that the C_{57} black mice had very few nodules even in the 2 x 300 (25 K.rad) immunised groups and there was little gross pathology.

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FIGURE 2-44

Mean recovery (MWR $\pm$ SD) N. dubius day 35 challenge infection in NIH, CFLP, C_{57} and C_{57}/NIH previously immunized with 2 x 300 normal (N) or 2 x 300, 25 K.rad (⚡) irradiated larvae

GROUP	STRAIN	IMMUNIZING LARVAE	CHALLENGE INFECTION
A	NIH	(⚡)	100Nd
B	CFLP	(⚡)	100Nd
C	C_{57}	(⚡)	100Nd
D	C_{57}/NIH	(⚡)	100Nd
E	NIH	(N)	100Nd
F	CFLP	(N)	100Nd
G	C_{57}	(N)	100Nd
H	C_{57}/NIH	(N)	100Nd
I	NIH	-	100Nd
J	CFLP	-	100Nd
K	C_{57}	-	100Nd
L	C_{57}/NIH	-	100Nd

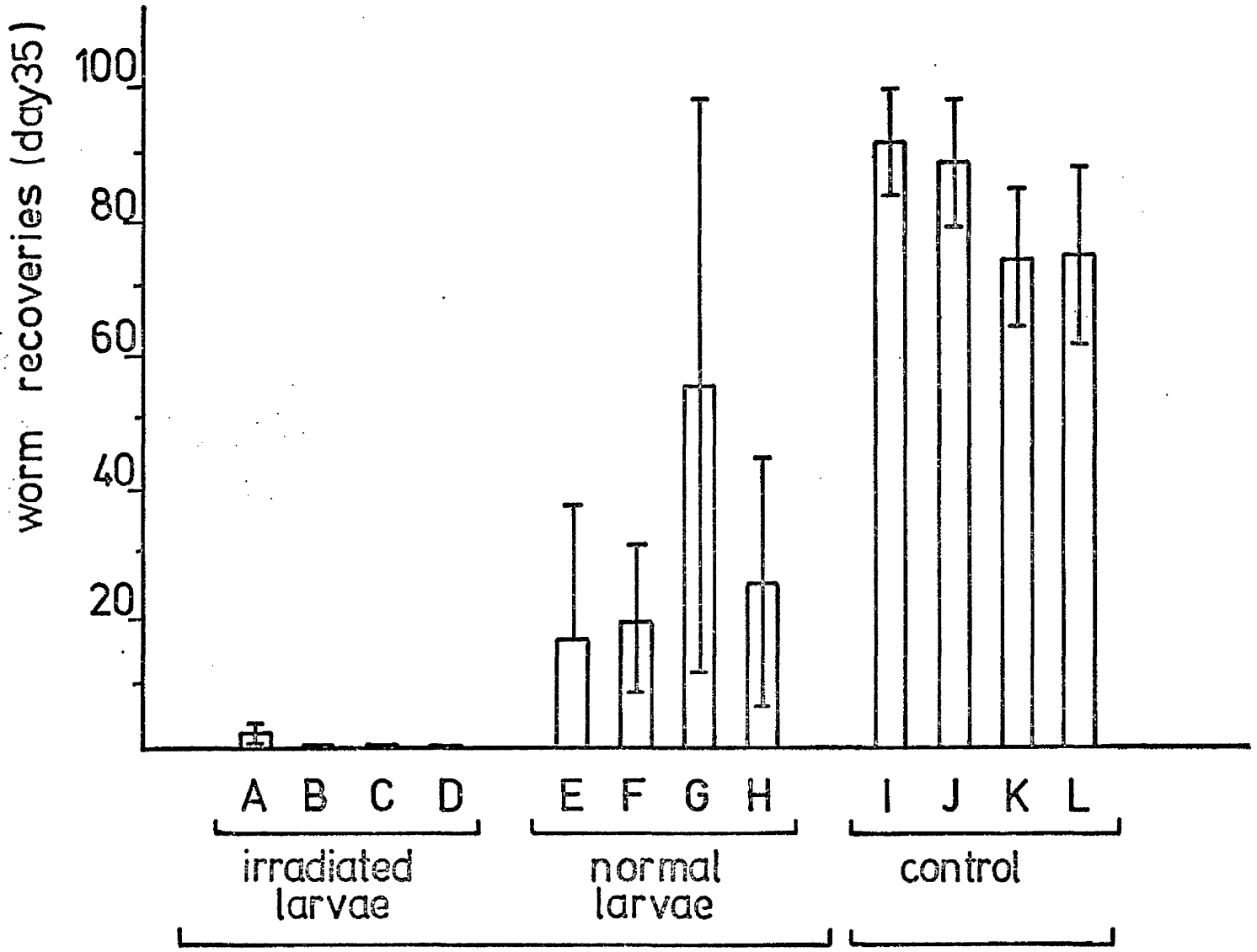
worm recoveries (day35)
100
80
60
40
20
A B C D
E F G H
I J K L
irradiated larvae
normal larvae
control
many large nodules on anterior small intestine except groups C and G, the C_{57} strain.
no nodules

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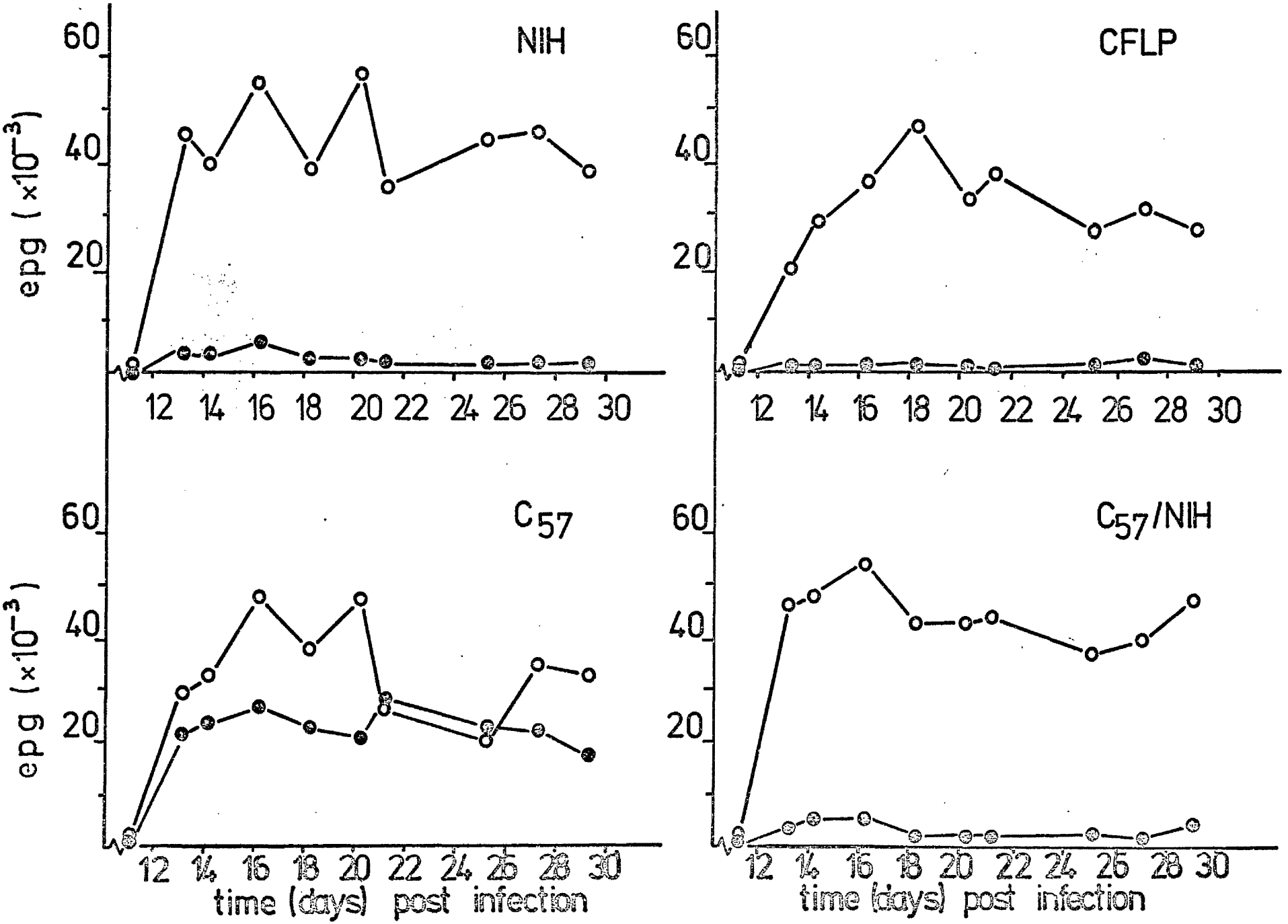
FIGURE 2-45

Egg counts (e.p.g. x 10^{-3}) from groups shown in Figure 2-44

- ● Mice immunized 2 x 300 normal larvae
- ○ Challenge infection control

No egg counts were recorded from mice immunized with 2 x 300, 25 K.rad irradiated larvae


NIH
CFLP
C57
C57/NIH
epg (x10⁻³)
60
40
20
12 14 16 18 20 22 24 26 28 30
time (days) post infection

None of the challenge infection control groups had nodules. Nodules are areas of inflammatory cell infiltration which form about developing N. dubius larvae whilst they are situated within the muscularis externa. As animals become immune larvae may become trapped within the nodules by the intense inflammatory reaction about them. The importance of this will be brought out in the discussion. This experiment showed that the protection obtained using irradiated larvae is not strain dependent and is therefore an inherent property of the irradiated larvae.

DISCUSSION

The results presented in this section of the Thesis have shown that there was no evidence for a loss of N. dubius from the intestine of NIH mice in the first forty days of primary infection, indicating that spontaneous cure does not occur with all nematode species. In order to raise immunity to challenge infection it is necessary to give multiple drug-terminated infections. The drug used here, Pyrantel embonate, was shown to be effective against adult worms in the intestine but did not affect the establishment and survival of subsequent infections. Mice given 6 immunizing infections of 100 larvae were more resistant to challenge infection than were mice given 3 times 200 larvae. Despite the high levels of protection in donors, serum transfers from mice immunized with multiple infections protected recipients but only to a small degree and were ineffective when administered after adult emergence. MLNC from mice immunized with multiple infections were more effective than serum in transferring immunity but again the effectiveness was not as great as that recorded in other systems or by other authors working with N. dubius. Failure to transfer high levels of immunity was not a property only of MLNC since SC were less effective.

The lack of resistance to a primary infection may be due to the immunosuppressive effects of the parasite, but attempts to boost the response of mice by use of an immunostimulant or by splenectomy were unsuccessful. The response to a challenge infection in splenectomised animals was also unaltered. The resistance of worms to intestinal inflammatory changes induced by T. spiralis expulsion showed that surgically implanted N. dubius adult worms are susceptible to these changes and can be expelled from the intestine.

A comparison of immunity to and cross immunity between laboratory and field strains of N. dubius was undertaken. It was found that the field strain of N. dubius isolated from Apodemus sylvaticus was able to develop to the adult stage only in immunosuppressed laboratory mice. Mice immunized with a field strain infection were resistant to challenge with laboratory strain larvae. This protection was greater than that obtained with an immunizing infection of laboratory strain parasite. Mice immunized with a field strain infection during immunosuppressive treatment were not resistant to a subsequent infection of laboratory strain parasites.

Stimulation of immunity to N. dubius in NIH mice normally requires multiple immunizations over a lengthy time period. A method of stimulating immunity which has been successful in other systems and does not require multiple infections is that of using irradiation attenuated parasites. The development of nematode parasites can be affected by irradiation in such a way that the host is able to destroy larvae which would normally escape the immune response. Before using irradiated larvae to stimulate immunity it was necessary to examine the effect which irradiation (with Cobalt 60) had on the parasite. The effect of irradiation was dose dependent. At 7.5 K.rads egg production was abolished but worm numbers were unaffected. Irradiation at 10 K.rads or above caused a reduction in worm recoveries and it was shown by CA treatment of infected mice that this reduction was due to the effect of irradiation and not to the immune response of the host. The results in the previous section had shown that N. dubius has an immunosuppressive effect on the response of mice to T. spiralis. Irradiated larvae (15 K.rads) were also capable of causing a delay in the rejection of T. spiralis from NIH mice.

Larvae irradiated at 10-30 K.rads induced high levels of protection to a challenge infection in NIH mice and down to a lower limit of 100 larvae this stimulation of protection was dose independent. The protection against challenge infection was evident when mice were challenged 17 or 45 days after the immunizing infection. The high level of protection obtained with irradiated larvae was not restricted to the high responder NIH mice, since CFLP, C_{57} and C_{57}/NIH mice responded equally well to immunization with irradiated larvae.

The experiments reported here have shown that N. dubius can survive in NIH mice for up to 40 days post primary infection. Throughout this time the sex ratio of parasites recovered at autopsy remained close to 1:1, a result in keeping with that of Cypess, Lucia, Zidian and Rivera-Ortiz(1977) who observed that in LAF_1 mice, a strain in which primary infection adult worm expulsion does occur, the sex ratio was maintained even during the period of worm expulsion from the gut. N. dubius primary infections have been reported to survive as long as eight months (Ehrenford 1954) and in this laboratory infections lasting as long as five months have been recorded.

Excluding the contribution of any immunosuppressive effect of N. dubius, the long term survival of the parasite in NIH mice is difficult to explain, since this strain efficiently eliminates other intestinal helminths, often more rapidly than other mouse strains (Wakelin, 1975, Wakelin and Lloyd 1976). Day, Howard, Prowse, Chapman and Mitchell (1979) have suggested that N. dubius may be less dependent upon the functional integrity of its ES-products than are other nematodes and also that the adult worm may be more resistant to those non-specific (presumably inflammatory) components of the intestinal expulsion mechanism which effectively eliminate other parasites. However, as was mentioned earlier, (see General Introduction) intestinal

inflammatory changes are not a prerequisite for the expulsion of all helminths. Cypess et al (1977) could find no histological changes in the intestinal villi adjacent to N. dubius adult worms in LAF_1 mice when the expulsion process was underway. It is possible that the host was able to damage the parasite whilst it was in the tissue, in the first 8 days of infection and that as a result, the adult worm was unable to survive. The lack of histological change about the worms makes it unlikely that the expulsion was caused by antibody damage to the worm followed by the action of a cell component (Ogilvie and Love 1974, Wakelin 1975, Behnke and Parish 1979).

There are a number of ways in which resistance to re-infection with N. dubius might be expressed:

1. Self-cure i.e. rapid expulsion of residual adult stages following ingestion of infective larvae (Cypess and Van Zandt 1973, Cypess and Zidian 1975)

2. Reduction in the number of larvae that penetrate and develop in the intestinal tissue (Panter 1969a, Behnke and Parish 1979)

3. Trapping and destruction of larval stages during development in the mucosa (Van Zandt 1961, Chaicumpa, Prowse, Ey and Jenkin 1978, Behnke and Parish 1979)

4. Retardation in larval development resulting in delayed emergence of pre-adult stages (Van Zandt 1961, Behnke and Parish 1979)

5. Reduction in size of adult stages (Panter 1969a, Jones and Rubin 1974, Behnke and Parish 1979)

6. Expulsion of adult stages following emergence (Cypess et al 1977a, Behnke and Parish 1979, Jacobson, Brooks and Cypess 1980).

Cypess and Van Zandt (1973) and Cypess and Zidian (1975) have shown that in S-W mice after 3, spaced oral infections a subsequent challenge with larvae results in the elimination of the pre-existing adult worm population (self-cure) and the failure of the challenge larval infection to develop to the adult stage (protection). Other strains tested did not show the self-cure reaction but did prevent development of the larval challenge to the adult stage (Cypess and Zidian 1975). The basis of the self-cure response against N. dubius was not investigated and the length of time after immunization over which such a reaction was possible was not studied and so an immediate hypersensitivity reaction cannot be excluded. Panter (1969b) and Jones and Rubin (1974) did provide some evidence to show that an immediate type hypersensitivity reaction may prevent larval establishment. Panter (1969b) induced a systemic anaphylactic reaction to horse serum and prevented larval establishment thus providing an obvious role for serum factors in immunized mice. Bartlett and Ball (1974) claimed that third stage larvae could be found free in the lumen of the small intestine in immune mice up to 14 days after challenge and this they attributed to the effect of antibodies. Although no mechanism was proposed they suggested that their findings offered support for the results of Panter (1969b).

The serum transfer experiments reported here gave disappointing results in that the peak protection achieved was only 26%. Other authors have had a similar lack of success (Chaicumpa, Jenkin and Rowley 1976, Cypess 1970, Panter 1969). In contrast Behnke and Parish (1979) have demonstrated that serum factors do play a role in protection

against N. dubius. Transfer of immune serum caused stunting of worms and a reduction in worm fecundity, but was only effective when the serum was administered before day 6 of infection. No effect was found when serum was administered after the parasites had emerged from the intestinal tissue. There was an early expulsion of larval stages from serum recipients, as worm counts in treated groups were always lower than those of controls. However the most surprising finding was that serum recipients rejected almost all adult worms after the fourth week of infection. This delayed rejection led the authors to conclude that a second component was involved in the expulsion process, the action of which had been facilitated by the immune serum. Such a dual mechanism for worm expulsion has been proposed for the elimination of N. brasiliensis from rats (Ogilvie and Love 1974, Love 1975). These authors envisaged that after antibodies had damaged worms a second, cellular component of the immune response was responsible for mediating worm expulsion. However, with N. brasiliensis in the mouse it was found that passively immunized animals did not eliminate the parasite and Ogilvie (1971) suggested that this was because the second step had not been induced. Behnke and Parish (1979) assumed that the effect of immune serum against N. dubius, when administered early in infection, resulted from a direct action on the developing larvae and the prevention of the action of immunosuppressive parasite factors. Failure of serum transferred later in infection was attributed to an already compromised host immune system or to the inaccessability of adult worms in the gut lumen. Both suggestions would fit well the data presented here. Further evidence for the involvement of serum factors in immunity to N. dubius is the correlation of a peak in the serum IgG_1 response with the onset of complete immunity in mice (Prowse, Ey and Jenkin 1978), although how much of this IgG_1 was specific to N. dubius is not known. Cypess,

Ebersole and Molinari (1977) and Molinari, Ebersole and Cypess (1978) found that IgG_1 levels in intestinal perfusates and in serum increased over the first 7 days of a challenge infection in resistant animals (2 previous infections) and remained high till day 20, the end of the study. In non-resistant animals (1 previous infection) the increase occurred between days 7 and 14 after challenge. As much as 45% of the IgG_1 present was found to be specific anti-worm antibody by the quantitative antibody-binding method of Nash and Heremans (1969). Crandall, Crandall and Franco (1974) also examined humoral responses to N. dubius in mice. The early phase of infection was associated with a drop in IgG_1 levels before a significant increase occurred. However the significant increase did not happen in all experiments and this inconsistency detracts from their findings. Prowse, Mitchell, Ey and Jenkin (1978) in their study of the susceptibility of hypothymic (nude, nu/nu) Balb/c mice demonstrated that not only were IgG_1 levels low in Balb/c nu/nu but also in some of the Balb/c nu/nu T-cell reconstituted mice which were still not resistant to infection. Their explanation was that a full complement of T-cells was essential for the production of a high IgG_1 response and that such a response was important in mediating complete protection. A high IgG_1 level may have been responsible for the effectiveness of the immune serum transferred early in infection (Behnke and Parish 1979) and in the results presented here. Many authors have reported successful, and unsuccessful attempts to passively transfer immunity with serum, against a number of nematodes (see General Introduction). On occasions protective serum fractions have contained IgG_1 (Jones, Edwards and Ogilvie 1970) and large numbers of IgG_1 producing cells have been found in the mesenteric lymph node of T. spiralis infected mice (Crandall and Crandall 1972), the organ in line for antigen capture (see Chapman et al 1979). Selby and Wakelin (1973) has speculated that IgG_1 would appear to have all the properties

essential for an immunoglobulin which could be involved in immunity to T. muris in mice. Chapman et al (1979) examined IgG_1 levels in M. corti and N. dubius infected mice and after using multiple high dose injections of SRBC as a model of chronic antigen exposure (such as occurs in N. dubius infection) concluded that the IgG_1 response arises from a T-cell dependent stimulation of B-cells. The T-dependence of the IgG_1 response is backed by the results of Prowse et al (1978) described earlier.

The failure to demonstrate the involvement of serum by means of passive transfer experiments may be due to the failure to induce cooperation with other components of the immune system and is probably related to the timing and collection and transfer of immune serum relative to infection. Chaicumpa, Jenkin and Fischer (1977) provided good evidence for the involvement of serum in immunity to N. dubius. They demonstrated that when N. dubius larvae were suspended in serum from immune mice (intravenously immunized) in millipore chambers and inserted into normal mice the cells from the normal mice were unable to damage (reduce infectivity) of larvae within the chamber although the cells were able to contact the larvae. In contrast cells from immune animals did damage larvae, but trypsin treatment abolished this activity and the presence of a specific cytophilic antibody on the surface of these cells was proposed (Chaicumpa and Jenkin 1978). This, together with the results of Behnke and Parish (in press) points towards an important role for serum factors in the immune response against N. dubius in mice.

CELL TRANSFERS AND FIELD STRAIN LARVAE

Successful transfers of immunity against N. dubius have been reported by Cypess (1970) and Behnke and Parish (1981, in press) using SC and MLNC respectively. The experiments reported here, in which

similar procedures were used to immunize the cell donors did not produce the levels of protection reported by these authors. In both experiments MLNC were more effective in transferring immunity than were SC a result which is not surprising since the MLN forms a major part of the GALT. There are a number of possible explanations for this relative lack of success, perhaps the most likely one being the timing of challenge infection in relation to cell transfer. In the present work mice were challenged on the same day as they were given cells and this may have exposed the cells to 'necrosin' or some other parasite product which could have prevented their action. Cypess (1970) and Behnke and Parish (1981 in press) delayed infection for several days after cell transfer and thus the cells may have been less susceptible to such factors and able to cooperate with host cells to mount a rapid, effective immune response.

The relative lack of success with the cell transfer experiments led to the experiments in which immunostimulation was attempted. The aim behind this aproach was to boost the activity of the monocyte-macrophage series since macrophages are thought to be involved in the response against infections of N. dubius (Chaicumpa, Jenkin and Fischer 1977). The Coumarin treatment failed to cause any change in infection characteristics in terms of worm trapping in the intestinal tissue or in worm expulsion up until day 20. Macrophage activity during infection was examined in earlier work (see Section 1(2)) but no check was made to determine if Coumarin did in fact activate macrophages. However, if as the results of the acid-phosphatase study suggest, macrophages in NIH mice are already highly active then the Coumarin may not have been able to boost this activity. The second attempt at enhancing mono-nuclear phagocyte activity was by splenectomy. Splenectomy is known to produce greatly enhanced phagocytosis by

macrophages and it has been suggested that this is due to the removal of a suppressor T-cell population, or its product (Skamene, Chayasirisobhan and Kongshavn 1978). In both experiments reported here there was no difference in egg counts or mean adult worm recovery from control and splenectomized mice after primary and secondary infection. These results agree with those of Baker (1955) who showed that splenectomized mice given 2 x 400 N. dubius infective larvae harboured the same number of worms at autopsy as did mice given 1 x 800 larvae, i.e. splenectomy had no effect on resistance to reinfection.

The results obtained by Behnke and Parish (1979) in their work on the passive transfer of immunity with serum and those of Jacobson, Brooks and Cypess (1980) indicate that adult N. dubius can be expelled from the mouse intestine. However, loss of worms from the intestine has not been recorded in this laboratory.

Day et al (1979) have suggested that N. dubius is more resistant than other parasites to intestinal inflammation. The capacity of N. dubius to resist inflammatory changes was tested by exposing N. dubius to the inflammation which was generated by a prior infection with T. spiralis. Adult N. dubius worms were transferred by laparotomy at various times after this infection. The results provide clear evidence that although adult N. dubius did establish in the intestine after transfer the worms were subsequently rejected by the inflammation induced by T. spiralis which is thought to be non-specific in its action. No evidence exists to show antigenic cross-reactivity between T. spiralis and N. dubius and since the N. dubius adult worms were established for only a few days it is unlikely that they would have generated a response effective against themselves in such a short time. These results would support the hypothesis that the final step in worm expulsion can be a non-specific event (see Discussion,

Kennedy 1980) induced by changes in the intestinal environment which make parasite survival impossible. However, Cypess et al (1977) could find no histological changes in the intestines of LAF_1 mice during the normal expulsion of N. dubius and this suggests that even although inflammation may have caused expulsion of N. dubius in the experiments reported in this study, it may not be an essential requirement for worm expulsion. The host may have a variety of immune mechanisms which can operate against intestinal parasites. If one mechanism is compromised another may come into action with the same result.

The use of N. dubius field strain was intended to provide some information of possible strain differences in infection characteristics within the species N. dubius. Strain differences in infection characteristics have been recorded by other authors and it is possible that long term passage in the laboratory may have selected for a strain which is relatively resistant to the host immune response. Such a possibility is an important consideration in studies on resistance and might explain the long-term survival of the parasite in laboratory mice.

Evidence for changes in N. dubius after only a few passages through laboratory mice has been provided by the work of Dobson and Owen (1977), who showed that the numbers of N. dubius which established in Quackenbush (Q) mice increased with the number of passages through this strain.

The strain of parasite maintained in this laboratory is equivalent to the subspecies Heligmosomoides polygyrus bakeri, according to the criteria of Durette-Desset, Kinsella and Forrester (1972), Behnke and Parish (1979). H. p. bakeri is thought to have arisen

from the Old World strain Heligmosomoides polygyrus polygyrus, a parasite of murid rodents of the Genera Mus and Apodemus (Durette-Desset et al 1972, Forrester and Neilson 1973). The field strain of N. dubius used in this study is therefore likely to be H. p. polygyrus.

The first experiment demonstrated that despite immunosuppressive treatment of the host, the field strain of N. dubius was unable to survive at the numbers and with the egg production levels associated with a laboratory strain infection in laboratory mice. The results of these infections can be explained in two ways:

1. the field strain infection is more immunogenic than the laboratory strain parasite,

or 2. the parasites are equally immunogenic but laboratory strain parasites are able to circumvent the response whereas field strain parasites are not.

The challenge infection with laboratory strain infection demonstrated that although the two parasites have remarkably different infection characteristics in NIH mice they apparently share common (cross-reacting) antigens since the field strain infection provided a high level of protection against a laboratory strain challenge. In fact greater protection than a homologous immunizing infection. Retention of field strain larvae in the intestinal tissue would have resulted in a greater exposure of the host to larval antigens and may be the reason for the protection but these larvae may have been more immunogenic than laboratory strain larvae. The retention of the field strain larvae within the tissues makes it impossible to decide between these two hypotheses.

Dobson and Owen (1977) pointed out that the increase in infectivity of the parasite after ten serial passages through Q mice was specific for Q mice since C_3H became more refractory to infection.

They also showed an apparent increase in immunogenicity of the parasite since the numbers of worms expelled by day 28 from Q and C_3H was increased. The increased expulsion was attributed to the selection of a genetically homogeneous population. The long term infections obtained with N. dubius in this laboratory suggest that enhanced immunogenicity has not occurred or that the parasite avoids the subsequent host response. The results of Dobson and Owen (1977) do not fit the hypothesis that the host-parasite relationship evolves towards specific unresponsiveness of the host in the presence of parasite antigens or a reduction in antigenic stimulation by the parasite. The relationship of N. dubius in NIH mice does appear to fit this hypothesis. Various authors have reported on changes in parasite infectivity after serial passages through the same or different host species. There seems to be no agreement about whether the parasite loses or retains infectivity for the original or for other hosts (compare results of Solomon and Haley (1966) on N. brasiliensis, with those of Wescott and Todd (1966) with the same parasite, and Forrester and Neilson (1973) on N. dubius).

IRRADIATED LARVAE

The earlier parts of this section have described the results of three methods of immunizing mice against N. dubius. These were:

1. multiple infection with normal larvae

and 2. i/p injection of exsheathed larvae,

both forms of homologous immunization, and

3. heterologous immunization using infections with a field strain of N. dubius.

The major drawbacks of these methods are that they are time consuming and require large numbers of larvae or that in the case of heterologous immunization larval availability is strictly limited. Other methods of immunization usually involving the use of larval antigen preparations

have been tried with varying degrees of success (Van Zandt 1962, Cypess 1970, Rubin, Leuker, Flom and Andersen 1971). Irradiation attenuated parasites have been used with a great deal of success in a number of systems but have not been tried with N. dubius. Miller (1967) has outlined the general principles involved in the use of irradiated larval vaccines and has emphasized the need to establish the characteristics of infection with irradiated larvae at various dose levels of irradiation. This is particularly important in the clinical situation where selection for radio-resistant strains must be avoided. The first three experiments were designed to assess the effect of various doses of irradiation on the growth and survival of the parasite.

The level of irradiation chosen in the first experiment, 6.5 K.rads, is known to have some effect on the normal development of other intestinal helminths. The results presented here were in agreement with those of Behnke, Parish and Hagan (in press) in that egg production by female worms was all but abolished. There are two possible reasons for this. The obvious one is that irradiation had a direct effect on the female worms; this effect is probably responsible for most of the reduction in egg counts. However irradiation could also act against male worms and thus have an indirect effect on female egg production. Authors working on other systems have recorded that male worms are more susceptible to the effects of irradiation than are female worms and that this holds for N. dubius has been demonstrated by Behnke et al. There was a significantly decreased worm recovery from the mice given 6.5 K.rads irradiated larvae in the first experiment but such a reduction, at this dose level, was not recorded in subsequent experiments.

These later experiments demonstrated that irradiation at 15 K.rads, and above, damages the parasite preventing it from emerging from the mucosa. The results of Behnke et al indicate that the numbers of worms recovered from mice starts to be reduced at levels of irradiation from 10 k.rads upwards. It might be assumed that the larvae are then eventually overcome by the hosts response but the evidence presented here indicates this is not the cause of the reduced numbers of worms recovered from mice given irradiated larvae since immunosuppressive treatment (with CA) did not increase worm recoveries. The CA was administered rather late in the infection and may not have had any great effect. However, Behnke et al have obtained the same result when CA was given every two days from the day of infection.

In the experiments presented here, and in those of Behnke et al. irradiation of worms had its main effect within the tissues of the host, since the larvae failed to develop and emerge into the intestinal lumen. The parasites which did emerge were capable of surviving until at least day 35 of infection and there was no evidence of a further loss of worms over this time period. Behnke et al. have shown that the subsequent survival of irradiated parasites i.e. after five weeks post infection, is affected and that these worms are lost from the intestine.

The work presented in the paper will not be discussed again but it is perhaps useful to be reminded of the important points. The most important variable in stimulating immunity with irradiated larvae was the dose of irradiation used. Levels of 10-30 K.rads were equally effective.

Provided this dose of irradiation was used the number of immunizing doses was unimportant. The prolonged exposure to larval antigens

which the host receives as a result of infection with irradiated larvae is probably the most important factor in conferring the strong immunity to re-infection. Behnke and Wakelin (1977) have provided evidence to show that when the host is exposed to a divided infection, which exposes it to larval stages for a considerably longer period of time than does a single infection, the immunity evoked is much greater.

The experiments following those presented in the paper have demonstrated that down to a lower limit of 100 larvae the number of irradiated larvae administered is not important in determining the level of protection to subsequent challenge. Panter (1969a) provided evidence that a single infection of 50 normal larvae was as effective as 200 normal larvae in stimulating immunity to challenge. Single and multiple infections have already been compared (Experiment 57) but in that experiment the challenge infection used to assess the level of immunity was administered within 16 days of the last immunizing infection. As a result, the challenge infection of normal larvae may have been caught up in the response which was already underway against the immunizing infections. To test whether the immunity induced by irradiated larvae lasted beyond this time a delayed challenge was given to mice which had been immunized with a single dose of irradiated larvae (Experiment 59). The results showed that even when the challenge infection with normal larvae was delayed for 45 days the level of protection was not only as high as when the challenge infection was given 17 days after immunization but also as high as in mice that had been given 3 doses of irradiated larvae. Miller (1965) has shown that immunity to A. caninum, after vaccination with irradiated larvae, persists for at least seven months and is not enhanced by booster infections during this period. Although in the present studies

the period between immunizing and challenge infections was only 45 days there was no evidence of waning of immunity with time.

The immunosuppressive effect of N. dubius which delayed the expulsion of concurrent infections of T. spiralis from mice was still operative when the N. dubius larvae had been irradiated at 15 K.rads. This evidence favours the induction of the immunosuppressive action by the larval stage but also suggests that the immunosuppression caused by the parasite can not be wholly responsible for the limited immunity obtained after a single infection of normal larvae. This interpretation assumes of course that the mechanism which delays T. spiralis expulsion is the same as the mechanism which aids the survival of N. dubius. Larvae irradiated at 15 K.rads still produced a strong immunity to challenge infection with normal N. dubius larvae despite the failure of many of the larvae in the immunizing dose to complete development. The stage at which development is affected by irradiation is not known precisely.

Irradiated larvae were used in this study in the hope that they would provide a quick and effective method of immunizing mice against N. dubius with the aim of being able to transfer this immunity to naive mice with cells and serum. The one experiment (Experiment 60) in which this was attempted did not give clear-cut results. The possibilities that the cells essential for effective transfer of immunity were absent or present only in low numbers in the MLN cannot be excluded. Wakelin and Lloyd (1976b) demonstrated that cells taken from donors that had been multiply immunized against T. spiralis did not transfer high levels of immunity against this parasite. It was suggested that the numbers of memory cells (anti-T. spiralis) present in the MLNs of these mice would not be any greater after multiple immunization than after a single infection. Equally possible is the

suggestion, given earlier, that the cells were exposed to parasite factors which prevented their action. The value of irradiated larvae in stimulating a transferrable immunity has yet to be realised but the result from Group B (Experiment 60) indicates that the cells do have some activity and that once the timings of infections and transfers have been investigated irradiated larvae may well prove to be very useful.

Intraspecific variation in host responsiveness has been recorded in a number of host-parasite systems (see Review, Wakelin 1978) and has also been documented for N. dubius in mice (Spurlock 1943, Liu 1966, Zidian 1975 [M.Sc. quoted Cypess et al 1977] and Prowse et al 1979). With increasing interest in the genetic control of immune responses and the availability of congenic mice it has become apparent that genetically determined differences may provide a key to the understanding of the immune/inflammatory mechanisms responsible for parasite elimination. Wakelin (1980) and Wakelin and Donachie (1980) have already made use of genetically defined host strains for their seminal work with T. spiralis in the mouse.

Variations in responsiveness to N. dubius cover a complete spectrum, ranging from the chronic infections which occur in NIH mice to the short term infections, ending in worm expulsion, which occur in LAF_1 mice (Cypess et al 1977). The reason(s) for these differences in responsiveness are not known and it is also not known if the immunosuppressive action of N. dubius so obvious in NIH mice during infection also occurs in LAF_1 mice or in other mouse strains.

The existence of genetically determined variations in responsiveness is an important factor that must be considered when a vaccine is to be used in a clinical situation and it is therefore an important

aspect to study in experimental systems, such as the use of irradiated larvae in stimulating immunity to N. dubius. The study described here was intended to determine if the response evoked by irradiated larvae was in some way associated with the rapid- responder status of the NIH mice that had been used in the earlier studies.

The results were extremely interesting. In all the strains tested two doses of irradiated larvae gave solid protection against a challenge infection, as assessed by egg counts and by mean adult worm recoveries on day 35 post challenge. Therefore it can be concluded that the response to attenuated parasites is strain independent and is related to an inherent property of irradiated larvae. However, perhaps the most interesting result was the intraspecific variation measured after immunization with two doses of normal larvae. NIH, CFLP and C_{57}/NIH mice given this immunizing schedule were all reasonably well protected against a challenge infection, although the extent of this protection, about 80 % was not as great as that produced by irradiated larvae. C_{57} Black mice on the other hand did not respond well to 2 doses of normal larvae and this was reflected in the levels of protection against challenge infection, which averaged about 50%. As C_{57}/NIH mice responded as well to two doses of normal larvae as did NIH mice it can be concluded that responsiveness is inherited as a dominant characteristic. The fact that C_{57}Black mice responded as well to immunization with irradiated larvae as did the other strains of mice suggests that there may well be some threshold requirement of antigen exposure required to stimulate protection. This threshold may have been exceeded for all the strains in this study by 2 doses of irradiated larvae but not in C_{57} by 2 doses of normal larvae. What mechanism may underlie this threshold effect in C_{57} mice? One possible explanation is that the response initiated by

irradiated larvae in the gut tissue is much more intense and persistent than that stimulated by normal larvae and, as a result, the invading challenge infection is simply caught up in this response. However evidence from earlier experiments with irradiated larvae in NIH mice suggests that the time of challenge after immunization with irradiated larvae is not important in determining the magnitude of the response to challenge. When NIH mice were challenged at 17 or 45 days post immunization protection was still greater than 90%. Although there is no data on this point for C_{57} mice, it seems likely that immunity to challenge was not simply due to a persistent response from the immunizing infections. Therefore it may be that a higher threshold of immune stimulation is required to trigger an effective response in C_{57} mice. This might explain the differences in the number of normal infections required to immunize different strains of mice.

Wakelin (1980) and Wakelin and Donachie (1980) have suggested that the failure of C_{57}Black mice to expel T. spiralis as rapidly as do NIH mice is due to their slowness in translating the immune stimulation of lymph node cells into the effector, inflammatory response which expels adult worms. Since macrophages and eosinophils apparently play an important role in the granulomatous reaction (nodules) about N. dubius in the gut it may be the processes involved in arming and triggering these cells which are defective in C_{57}Black mice. At the day of autopsy (day 35) in this experiment there was no evidence of any nodule formation in the C_{57}Black mice even in the mice immunized with irradiated larvae so it may be that a separate mechanism is involved in the elimination of N. dubius.

In many of the cases where irradiated larvae have been used as a means of stimulating a protective immune response there has been some

controversy as to whether or not the irradiated larvae are more immunogenic than normal larvae i.e. whether larva for larva they produce a better response than do normal larvae. It is difficult to imagine that the irradiation used in this study actually changed the antigenic structure of N. dubius. However, because of the damage to the development of the parasite it is possible that the quantity rather than the quality of the protective antigens available to the host, and hence protection, was increased. Agyei and Catty (1980) have suggested that this occurs with T. spiralis. Larvae given low doses of irradiation apparently produce more antigenic material than do normal larvae. Another and perhaps more feasible explanation is that irradiation delays development and maturation of the parasite allowing time for the host to mount an effective response. As a result the larvae are destroyed in the tissue and the host is exposed to a greater amount of antigenic materials, including somatic antigens, resulting in increased protection. Support for this comes from the fact that multiple immunization schedules, which extend the duration of host exposure to larval antigens, also produce greater protection than do single large doses of larvae.

There may not be any single reason why different mouse strains require different levels of stimulation to produce the same protective response. It is known that the immune response is under genetic control at all points between induction and expression and further more is subject to the genetically controlled modulating activity of helper and suppressor cell populations.

No matter what the reason is, the double immunization with irradiated larvae (and perhaps even a single immunization) is enough to protect all strains tested against a subsequent challenge infection with normal larvae indicating that, with the appropriate stimulation genetic variations in immune responsiveness can be circumvented.

SUMMARY DISCUSSION

The majority of laboratory models of intestinal helminth infection used today are those in which parasite elimination from the host occurs within 3 to 4 weeks of infection and these models have provided much of the available data on the immune mechanisms responsible for the rejection of intestinal parasites. In recent years models of chronic infections which perhaps reflect more accurately the types of infection important in man and his domestic stock have received more attention.

In the first section, (1(1) and 1(2)), these two types of infection were combined and the effect of N. dubius on the elimination of T. spiralis suggested that N. dubius had an effect on the lymphoid component of the immune mechanism which eliminates T. spiralis. Previous reports of the immunosuppressive effect of N. dubius (see Table 3) have not made any attempt to examine the competence of the cells involved in the response against the concurrently administered heterologous antigen. It was not clear how this effect on T. spiralis-reactive lymphocytes was caused, but the presence of N. dubius influenced both sensitization and expression of immunity and was apparent in the major classes of lymphocyte, B and T. Concentrated ES-products were not cytotoxic for lymphocytes although the effect of these products on mitogen responsiveness of cells was never assessed.

The change in the homing patterns of lymphocytes in the mice infected with T. spiralis and N. dubius may have been partly responsible for the delayed expulsion of T. spiralis but there are a number of important points which have to be considered before this conclusion is accepted. The first is that mice given immune MLNC (anti-T. spiralis) prior to a T. spiralis infection do not show enhanced

homing patterns and yet have a good immunity to challenge infection so enhanced homing may not be essential in the immune elimination of T. spiralis. Secondly, N. dubius infected mice did not show enhanced homing patterns at any time during infection, although this was assessed only till day 16, more important however, is that there was no evidence of decreased homing in these mice. Only the enhanced homing as a result of the T. spiralis infection was delayed. The change in the homing patterns could be a measure of N. dubius induced changes in the microenvironment of the small intestine which delay the onset of the inflammatory changes that finally eliminate T. spiralis. However, the attempt to measure the ability of mice to respond to an inert filter was not altered during an N. dubius infection although, once again there are some limitations on this interpretation.

Certainly the depressed lymphocyte responses may be one of the reasons why N. dubius survives for such a long period in NIH mice but depressed responses of lymphocytes from mice infected with other nematodes have been demonstrated here and in the work of Ljungstrom (1980). Other reasons must also be considered. There has also no doubt been selection for a strain of N. dubius which is resistant to the attempts of the host to bring about its elimination and it may be, as suggested by Dobson and Owen (1977) that N. dubius is susceptible to even slight selection pressures. Day et al (1979) have suggested that N. dubius may be insensitive to inflammatory changes in the host's intestine and so may be able to avoid expulsion. The results presented here show clearly that if there are severe inflammatory changes in the intestine, such as those induced by T. spiralis infection, then N. dubius will be eliminated. From previous experience this does not occur when high numbers 200-300 N. dubius are

present and the immunosuppressive effect of N. dubius may be responsible for the continued survival of N. dubius after elimination of T. spiralis.

Unfortunately it is not possible to make a definitive statement on the mechanism which causes the immunosuppression during N. dubius infection. Changes in homing patterns may be important as far as responses to intestinal helminths and other intestinal antigens are concerned but may not be responsible for the depressed responses to some of the heterologous antigens mentioned earlier (see Table 3). The contribution of antigenic competition to the immunosuppression may be large. Day et al (1979) have shown that N. dubius produces a large number of ES-products and this antigenic overloading may reduce the response to the important protective antigens and also might explain the depressed responses to mitogens.

The second section of the thesis dealt mainly with attempts to stimulate and analyse the response to N. dubius. The relative lack of success with serum and cell transfers can only be attributed to the timings of the cell collections and transfers and infections. Behnke and Parish (in press) have shown that immunity to N. dubius can be transferred synergistically with cells and serum from multiply immunized donors. The failure to transfer immunity with MLNC from mice immunized with irradiated larvae was surprising but again the timings of the events may not have been quite right.

The field strain parasite which is believed to be Heligmosomoides polygyrus polygyrus showed infection characteristics in NIH mice which were markedly different from those of the laboratory strain H. polygyrus bakeri. The field strain infection survived, only just, in NIH mice but the most interesting point was that it conferred a

substantial degree of protection to challenge infection with laboratory strain. This indicates that there must be a considerable degree of common 'shared' antigens between these two parasite variants even though the laboratory strain infection is better adapted to laboratory mice. It would be interesting to passage the field strain parasite through laboratory mice for several generations to determine whether or not similar adaptation occurs. Although not recorded here evidence indicates that the field strain parasite survives at very low infection levels in A. sylvaticus. The magnitude of the response which was stimulated in NIH mice may therefore reflect the greater immunogenicity of the field strain larvae. Low level and trickle infections in NIH mice should also be examined.

As was concluded at the end of the discussion of section 2 the full experimental potential of irradiated N. dubius larvae has yet to be realised. The basic parameters of immunization have been established and the effectiveness is without question. The major factor in the stimulation of immunity is believed to be the trapping of irradiated larvae followed by their eventual elimination within the gut mucosa. Subsequent infections are then attacked within the tissue so that the self-cure response reported by other authors and the spontaneous-cure recorded in other systems become less important elements in host immunity. The speed with which cells can infiltrate the tissue surrounding the parasite within the muscularis externa is all important. Unless there is an effective response at this stage it appears that the parasite can escape to the relative safety of the gut lumen. When this occurs then those other mechanisms, mentioned above, may come into operation. In NIH mice and in the other strains used in this study they were not

operative. The evidence presented here suggests that further studies on immunity to N. dubius must consider the different levels at which immunity can be expressed. In order to do this a mouse strain such as the LAF_1 which shows spontaneous cure would be invaluable. Parallel studies in NIH and LAF_1 mice would yield important information on the response to N. dubius.

Histological changes should be re-examined. The apparent lack of histological change about N. dubius during expulsion in LAF_1 mice might indicate that the damage to the parasite was inflicted at an earlier stage and is only later expressed. However, if this is the case then hypotheses of non-specific inflammatory mechanisms of worm expulsion will have to be restricted in their application.

The findings with irradiated larvae present an ideal situation for the examination of the types of cell which are involved in the response against this parasite. They may also lead to a suitable model for the examination of the effect of irradiation on nematode parasites, perhaps yielding information on whether or not irradiated larvae are, worm for worm, more immunogenic than larvae.

The use of double infections of T. spiralis and N. dubius to investigate aspects of suppression provided some useful results but the very complexity of the system suggests that another approach has to be employed. An 'in vitro' culture system, assessing responses to a concurrently administered antigen is recommended. A renewed search for circulating immune complexes should be initiated. This was attempted here, (though not reported), using an anti-worm antiserum raised in rabbits, but the technique was crude and despite several attempts yielded no useful information.

Research on *N. dubius* has been slow to progress mainly because of the need to establish the basic background information which is readily available for other laboratory models of intestinal helminth infection e.g. *N. brasiliensis* and *T. spiralis*. Various laboratories have made significant contributions. It is hoped that this thesis has helped establish some basic parameters but also that the information contained herein will stimulate new and fruitful lines of research.

REFERENCES

Africa, C.M. (1931) Studies on the host relations of Nippostrongylus muris, with special reference to age resistance and acquired immunity. Journal of Parasitology 18, 1-13.

Agyei, M. and Catty, D. (1980) Effects of irradiation of Trichinella spiralis infective larvae. Abstract, British Society Immunology, November.

Alizadeh, H. and Wakelin, D. (1980) Rapid expulsion of Trichinella spiralis in mice. British Society for Parasitology: Proceedings 81, XXIX.

Asherson, G.L., Allwood, G.G. and Mayhew, B. (1973) Contact sensitivity in the mouse. XI. Movement of T blasts in the draining lymph nodes to sites of inflammation. Immunology 25, 485-494.

Babero, B.B. and Matthias, D. (1967) Protospirura peromysci n. sp. (Nematoda: Spiruridea) and Other Helminths from Peromyscus spp. in Nevada. Proceedings Helminthological Society of Washington 34, 255-261.

Baker, N.F. (1955) The pathogenesis of Trichostrongyloid parasites: Some effects of Nematospiroides dubius on the erythrocyte patterns and spleens of mice. Experimental Parasitology 4, 526-541.

Barth, E.E.E., Jarrett, W.F.A. and Urquhart, G.M. (1966) Studies on the mechanism of the self-cure reaction in rats infected with Nippostrongylus brasiliensis. Immunology 10, 459-464.

Bartlett, A. and Ball, P.A.J. (1974) The immune response of the mouse to larvae and adults of Nematospiroides dubius. International Journal for Parasitology 4, 463-470.

Baylis, H.A. (1926) On a Trichostrongylid Nematode from the Woodmouse (Apodemus sylvaticus). Annals and Magazine of Natural History 9, LViii 455-464.

Befus, A.D. and Bienenstock, J. (1979) Immunologically mediated intestinal mastocytosis in Nippostrongylus brasiliensis-infected rats. Immunology 38, 95-101.

Behnke, J.M. and Parish, H.A. (1979a) Nematospiroides dubius: Arrested Development of Larvae in Immune Mice. Experimental Parasitology 47, 116-127.

Behnke, J.M. and Parish, H.A. (1979b) Expulsion of Nematospiroides dubius from the intestine of mice treated with immune serum. Parasite Immunology 1, 13-26.

Behnke, J.M. and Parish, H.A. (1981) Transfer of immunity to Nematospiroides dubius: Co-operation between lymphoid cells and antibodies in mediating worm expulsion. Parasite Immunology (in press).

Behnke, J.M., Parish, H.A. and Hagan, P. (1981) The effect of gamma radiation on Nematospiroides dubius. Factors affecting the survival of worms in a primary infection in mice. Journal of Helminthology (in press).

Behnke, J.M. and Wakelin, D. (1977) Nematospiroides dubius: stimulation of acquired immunity in inbred strains of mice. Journal of Helminthology 51, 167-176.

Behnke, J.M., Wakelin, D. and Wilson, M.M. (1978) Trichinella spiralis: Delayed Rejection in mice concurrently infected with Nematospiroides dubius. Experimental Parasitology 46, 121-130.

Bell, R.G. and McGregor, D.D. (1979) Trichinella spiralis: expulsion in rats exposed to an abbreviated enteral infection. Experimental Parasitology 48, 42-50.

Brown, A.R., Crandall, R.B. and Crandall, C.A. (1976) Increased IgG catabolism as a possible factor in the immunosuppression produced in mice infected with Heligmosomoides polygyrus. Journal of Parasitology 62, 169-171.

Bryant, V. (1973) The Life Cycle of Nematospiroides dubius, Baylis, 1926 (Nematoda: Heligmosomidae). Journal of Helminthology XLVII, 263-268.

Camus, D., Dessaint, J.P., Fischer, E. and Capron, A. (1979) Nonspecific suppressor cell activity and specific cellular unresponsiveness in rat schistosomiasis. European Journal Immunology 9, 341-344.

Carlaw, C., Phillips, R.S. and Wakelin, D. (1980) Suppression of Babesia infections in mice infected with Trichinella spiralis. Laboratory meeting. Scottish branch. Transactions Royal Society Tropical Medicine Hygiene (in press).

Castro, G.A., Badial-Aceves, F., Smith, J.W., Dudrick, S.J. and Weisbrodt, N.W. (1976) Altered small bowel propulsion associated with parasitism. Gastroenterology 71, 620-625.

Castro, G.A., Hessel, J.J. and Whalen, G. (1979). Altered intestinal fluid movement in response to Trichinella spiralis in immunized rats. Parasite Immunology 1, 259-266.

Castro, G.A., Roy, S.A. and Stockstill, R.D. (1974) Trichinella spiralis: Peroxidase activity in isolated cells from the rat intestine. Experimental Parasitology 36, 307-315.

Chaicumpa, V. and Jenkin, C.R. (1978) Studies in vitro on the reaction of peritoneal exudate cells from mice immune to infection with Nematospiroides dubius with the infective third stage larvae of this parasite. Australian Journal Experimental Biology Medical Science 56, 61-68.

Chaicumpa, V., Jenkin, C.R. and Fischer, H. (1977a) The effect 'in vivo' of peritoneal exudate cells of immune and normal mice on the infectivity of the third stage larvae of Nematospiroides dubius. Australian Journal Experimental Biology Medical Science 55, 561-570.

Chaicumpa, V., Jenkin, C.R. and Rowley, D. (1976) Passive transfer of immunity to infection with Nematospiroides dubius from immunised mice to their offspring. Australian Journal Experimental Biology Medical Science 54, 245-252.

Chaicumpa, V., Prowse, S.J. and Jenkin, C.R. (1977b) Induction of immunity in mice to the nematode parasite Nematospiroides dubius. Australian Journal Experimental Biology Medical Science 55, 393-400.

Chapman, C.B., Knopf, P.M., Anders, R.F. and Mitchell G.F. (1979a) IgG_1 hypergammaglobulinaemia in chronic parasitic infections in mice: evidence that the response reflects chronicity of antigen exposure. Australian Journal Experimental Biology Medical Science 57, 389-400.

Chapman, C.B., Knopf, P.M., Hicks, J.D. and Mitchell, G.F. (1979b) IgG_1 hypergammaglobulinaemia in chronic parasitic infections in mice: magnitude of the response in mice infected with various parasites. Australian Journal Experimental Biology Medical Science 57, 369-387.

Chowaniec, W., Wescott, R.B. and Congdon, L.L. (1972) Interaction of Nematospiroides dubius and Influenza Virus in mice. Experimental Parasitology 32, 33-44.

Christie, P.R. (1979) The intestinal immune response of the mouse to the tapeworm Hymenolepis diminuta. Ph.D. Thesis University of Glasgow.

Clegg, J.A. and Smith, M.A. (1978) Prospects for the Development of Dead Vaccines against Helminths. Advances in Parasitology 16, 165-218.

Colwell, D.A. and Wescott, R.B. (1973) Prolongation of Egg Production of Nippostrongylus brasiliensis in Mice concurrently infected with Nematospiroides dubius. Journal of Parasitology 59, 216.

Connan, R.M. (1972) Passive protection with homologous antiserum against Trichostrongylus colubriformis in the guinea-pig. Immunology 23, 647-650.

Crandall, R.B. and Crandall, C.A. (1972) Trichinella spiralis: Immunologic Response to infection in mice. Experimental Parasitology 31, 378-398.

Crandall, R.B., Crandall, C.A. and Franco, J.A. (1974) Heligmosomoides polygyrus (= Nematospiroides dubius): Humoral and intestinal immunologic responses to infection in mice. Experimental Parasitology 35, 275-285.

Cross, J.H. (1960) The natural resistance of the white rat to Nematospiroides dubius and the effect of cortisone on this resistance. Journal of Parasitology 46, 175-185.

Cypess, R.H. (1970a) Demonstration of immunity to Nematospiroides dubius in recipient mice given spleen cells. Journal of Parasitology 56, 199-200.

Cypess, R.H. (1970b) Artificial production of Acquired immunity in mice by footpad injections of Crude Larval extract of Nematospiroides dubius. Journal of Parasitology 56, 320.

Cypess, R.H., Ebersole, J.L. and Molinari, J.A. (1977a) Specific Antibody Levels in the intestinal perfusates of Heligmosomoides polygyrus-infected mice. International Archives Allergy Applied Immunology 55, 496-503.

Cypess, R.H., Lubinieki, A.S. and Swidwa, D.M. (1974) Decreased susceptibility to Listeria monocytogenes in mice after infection with Trichinella spiralis. Infection and Immunity 9, 477-479.

Cypess, R.H., Lucia, H.L., Zidian, J.L. and Rivera-Ortiz, C.I. (1977b) Heligmosomoides polygyrus: Temporal, Spatial and Morphological Population characteristics in LAF_1/J Mice. Experimental Parasitology 42, 34-43.

Cypess, R.H. and Van Zandt, P. (1973) Time studies on the immune expulsion of Nematospiroides dubius in the mouse. Journal of Parasitology 59, 579-580.

Cypess, R.H. and Zidian, J.L. (1975) Heligmosomoides polygyrus (= Nematospiroides dubius): the development of self-cure and/or protection in several strains of mice. Journal of Parasitology 61, 819-824.

Day, K.P., Howard, R.J., Prowse, S.J., Chapman, C.B. and Mitchell, G.F. (1979) Studies on chronic versus transient intestinal nematode infections in mice. I. A comparison of responses to excretory/secretory (ES) products of Nippostrongylus brasiliensis and Nematospiroides dubius worms. Parasite Immunology 1, 217-239.

Denham, D.A. (1969) Immunity to Trichinella spiralis IV Passive Immunity in mice. Folia Parasitologia Praha 16, 183-187.

Despommier, D.D., MacGregor, D.D., Crum, E.D. and Carter, P.B. (1977) Immunity to Trichinella spiralis II. Expression of Immunity against adult worms. Immunology 33, 797-805.

Despommier, D.D. and Muller, M. (1970) Functional antigens of Trichinella spiralis. Journal of Parasitology 56, Section 2, Part 1, 76.

Dessaint, J.P., Camus, D., Fischer, E. and Capron, A. (1977) Inhibition of lymphocyte proliferation by factor(s) produced by Schistosoma mansoni. European Journal Immunology 7, 624-629.

Dineen, J.K. and Adams, D.B. (1971) The Role of the Recirculating Thymus-Dependent Lymphocyte in Resistance to Trichostrongylus colubriformis in the Guinea-pig. Immunology 20, 109-113.

Dineen, J.K. and Kelly, J.D. (1973) Expulsion of Nippostrongylus brasiliensis from the intestine of rats: the role of a cellular component derived from bone marrow. International Archives of Allergy and Applied Immunology 45, 504-512.

Dineen, J.K., Kelly, J.D., Goodrich, B.S. and Smith, I.D. (1974) Expulsion of Nippostrongylus brasiliensis from the small intestine of the rat by prostaglandin-like factors from ram semen. International Archives of Allergy and Applied Immunology 46, 360-374.

Dineen, J.K., and Wagland, B.M. (1966) The cellular transfer of immunity to Trichostrongylus colubriformis in an isogenic strain of Guinea-pig. II. The relative susceptibility of the larval and adult stages of the parasite to immunological attack. Immunology 11, 47-56.

Dobson, C. (1960) An investigation of the host-parasite relations and host specificity of Nematospiroides dubius, Baylis, 1926, Heligmosomidae, a mouse nematode in its normal and abnormal host. PhD. Thesis, University of Sheffield.

Dobson, C. and Owen, M.E. (1977) Influence of serial passage on the infectivity and immunogenicity of Nematospiroides dubius in mice. International Journal for Parasitology 7, 463-466.

Dobson, C. and Soulsby, E.J.L. (1974) Lymphoid Cell Kinetics in Guinea-pigs infected with Trichostrongylus colubriformis: Tritiated Thymidine uptake in gut and allied lymphoid Tissue, Humoral IgE and Haemagglutinating Antibody responses, Delayed Hypersensitivity Reactions and 'in vitro' Lymphocyte Transformations during primary infections. Experimental Parasitology 35, 16-34.

Durette-Desset, M.C., Kinsella, J.M. and Forrester, D.J. (1972) Arguments en faveur de la double origin des Nematodes nearctiques du genre Heligmosomoides Hall, 1916. Annales de Parasitologie (Paris) 47, 362-382.

Ehrenford, F.A. (1954) The Life Cycle of Nematospiroides dubius Baylis (Nematoda: Heligmosomidae). Journal of Parasitology 40, 480-481.

Eveland, L.K., Hsu, S.Y.Li., and Hsu, H.F. (1969) Cross-immunity of Schistosoma japonicum, S. mansoni and S. bovis in Rhesus monkeys. Journal of Parasitology 55, 279-288.

Fahmy, M.A.M. (1956) An investigation on the life cycle of Nematospiroides dubius (Nematoda: Heligmosomidae) with special reference to the free-living stages. Zeitschrift fur Parasitekunde 17, 393-399.

Faubert, G. and Tanner, C.E. (1975) Leucoagglutination and cytoxicity of the serum of infected mice and of extracts of T. spiralis larvae and the capacity of infected mouse serum to prolong skin allografts. Immunology 28, 1041-1047.

Feldmann, M., Basten, A., Boylston, A., Erb, P., Gorczynski, R., Greaves, M., Hogg, N., Kilburn, D., Kontiainen, S., Parker, D., Pepys, M. and Schrader, J. (1974) Interactions between T and B lymphocytes and accessory cells in antibody production. Progress in Immunology II, 3, 65-75.

Forrester, D.J. (1971) Heligmosomoides polygyrus (=Nematospiroides dubius) from wild rodents of Northern California: Natural infections, host specificity and strain characterisics. Journal of Parasitology 57, 498-503.

Forrester, D.J. and Neilson, J.T.McL. (1973) Comparative infectivity of Heligmosomoides polygyrus (= Nematospiroides dubius) in three species of Peromyscus. Journal of Parasitology 59, 251-255.

Frost, P. and Lance, E.M. (1974) The cellular origin of the lymphocyte trap. Immunology 26, 175-186.

Gabriel, B.W. and Justus, D.E. (1979) Quantitation of immediate and delayed hypersensitivity responses in Trichinella spiralis infected mice. International Archives Allergy Applied Immunology 60, 275-285.

Goose, J. (1975) Possible role of excretory/secretory products in evasion of host defences by Fasciola hepatica. Nature 275, 216-217.

Gordon, H.M. and Whitlock, H.V. (1939) A new technique for counting nematode eggs in sheep faeces. Journal Council Scientific and Industrial Research, Australia 12, 50-52.

Gore, R.W., Burger, H.J. and Sadun, E.H. (1970) Mechanisms of immunity in rats infected with Trichinella spiralis (Abs.). Second International Congress of Parasitology. Journal of Parasitology 56-57, 122.

Gowans, J.L. and Knight, E.J. (1964) The route of recirculation of lymphocytes in the rat. Proceedings Royal Society B. Biological Science 159, 257-282.

Griscelli, C., Vassalli, P. and McCluskey, R.T. (1969) The distribution of large dividing lymph node cells in syngeneic recipient rats after intravenous injection. Journal Experimental Medicine 130, 1427-1452.

Guy-Grand, D., Griscelli, C. and Vassalli, P. (1974) The gut-associated lymphoid system: nature and properties of the large dividing cells. European Journal of Immunology 4, 435-443.

Hall, J.G., Hopkins, J. and Orlans, E. (1977) Studies on the lymphocytes of sheep III. Destination of lymph borne immunoblasts in relation to their tissue of origin. European Journal Immunology 7, 30-37.

Hopkins, C.A. and Stallard, H.E. (1974) Immunity to intestinal tapeworms: the rejection of Hymenolepis citelli by mice Parasitology 69, 63-76.

Hudson, K.M., Byner, C., Freeman, J.C. and Terry, R.J. (1976) Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis. Nature 264, 256-258.

Humason, G.L. (1972) Animal tissue techniques. 3rd edition, W.H. Freeman and Co., San Francisco.

Jacobson, R.H., Brooks, B.O. and Cypess, R.H. (1980) Stages in the Life-Cycle Responsible for immunity to Nematospiroides dubius. American Society Parasitologists, 55th Annual Meeting, August 1980. (Abs.)

Jacobson, R.H. and Reed, N.D. (1974) The immune response of congenitally athymic (nude) mice to the intestinal nematode Nippostrongylus brasiliensis. Proceedings Society Experimental Biology and Medicine 147, 667-673.

Jacobson, R.H., Reed, N.D. and Manning, D.D. (1977) Expulsion of Nippostrongylus brasiliensis from mice lacking antibody production potential. Immunology 32, 867-874.

Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W., Sharp, N.C.C. and Urquhart, G.M. (1959) Immunological studies on Dictyocaulus viviparus infection in calves-double vaccination with irradiated larvae. American Journal Veterinary Research 20, 522-526.

Jarrett, W.F.H. and Sharp, N.C.C. (1963) Vaccination against Parasitic Disease: Reactions in vaccinated and immune hosts in Dictyocaulus viviparus infections. Journal of Parasitology 49, 177-189.

Jenkins, D.C. (1975) The influence of Nematospiroides dubius on subsequent Nippostrongylus brasiliensis infections in mice. Parasitology 71, 349-355.

Jenkins, S.N. (1977) Studies on the immune response of the mouse to the nematode Trichuris muris. PhD. Thesis, University of Glasgow.

Jenkins, S.N. and Behnke, J.M. (1977) Impairment of primary expulsion of Trichuris muris in mice concurrently infected with Nematospiroides dubius. Parasitology 75, 71-78.

Jenkins, S.N. and Wakelin, D. (1977) The source and nature of some functional antigens of Trichuris muris. Parasitology 74, 153-161.

Jones, C.E. (1974) Nematospiroides dubius: Mechanisms of host immunity II. Skin testing and immunosuppression of orally and subcutaneously sensitized mice. Experimental Parasitology 35, 453-464.

Jones, C.E. and Rubin, R. (1974) Nematospiroides dubius. Mechanisms of host immunity. I. Parasite counts, histopathology and serum transfer involving orally or subcutaneously sensitized mice. Experimental Parasitology 35, 434-452.

Jones, V.E., Edwards, H.J. and Ogilvie, B.M. (1970) The circulating immunoglobulins involved in protective immunity to the intestinal stages of Nippostrongylus brasiliensis in the rat. Immunology 18, 621-623.

Jones, V.E. and Ogilvie, B.M. (1971) Protective immunity to Nippostrongylus brasiliensis: the sequence of events which expels worms from the rat intestine. Immunology 20, 546-561.

Kassai, T., Redl, P., Jecsai, Gy., Balla, E. and Harangozo, E. (1980) Studies on the involvement of Prostaglandins and their precursors in the rejection of Nippostrongylus brasiliensis from the rat. International Journal for Parasitology 10, 115-120.

Keller, R. and Jones, V.E. (1971) Immunological and pharmacological analysis of the primary and secondary reagin response to Nippostrongylus brasiliensis in the rat. Immunology 21, 565-574.

Keller, R. and Keist, R. (1972) Protective immunity to Nippostrongylus brasiliensis in the rat. Central role of the lymphocyte in worm expulsion. Immunology 22, 767-773.

Kelly, J.D., Dineen, J.K. and Love, R.J. (1973) Expulsion of Nippostrongylus brasiliensis from the intestine of rats: evidence for a third component in the rejection mechanism. International Archives Allergy Applied Immunology 45, 767-769.

Kennedy, M.W. (1980) Immunologically mediated, non-specific interactions between the intestinal phases of Trichinella spiralis and Nippostrongylus brasiliensis in the mouse. Parasitology 80, 61-72.

Khoury, P.B., Stromberg, B.E. and Soulsby, E.J.L. (1977) Immune mechanisms to Ascaris suum in inbred guinea-pigs. I. Passive transfer of immunity by cells or serum. Immunology 32, 405-411.

Komatsu, T., Nishimura, T., Sano, R. and Shinka, S. (1979) Ascaris suum: Suppression of Reaginic and Haemagglutinating Antibody Responses in the mouse by Crude extract and Maintenance Fluid. Experimental Parasitology 47, 158-168.

Larsh, J.R. and Race, G.J. (1975) Allergic inflammation as a hypothesis for the expulsion of worms from tissues: a review. Experimental Parasitology 37, 251-266.

Levin, A.J. and Evans, T.C. (1942) The use of Roentgen radiation in locating an origin of host resistance to Trichinella spiralis infection. Journal of Parasitology 28, 477-483.

Liu, S.K. (1965) Pathology of Nematospiroides dubius I. Primary infections in C_3H and Webster mice. Experimental Parasitology 17, 123-135.

Liu, S.K. (1966) Genetic influence on Resistance of Mice to Nematospiroides dubius. Experimental Parasitology 18, 311-319.

Ljungstrom, I. (1980) Studies on the responsiveness of spleen cells to various polyclonal T and B cell activators during Trichinella spiralis infection. Parasite Immunology 2, 111-120.

Ljungstrom, I. and Ruitenberg, E.J. (1976) A comparative study of the immunohistological and serological response of intact and T cell-deprived mice to Trichinella spiralis. Clinical Experimental Immunology 24, 146-156.

Love, R.J. (1975) Nippostrongylus brasiliensis infection in Rats. Both antibodies and sensitised cells are necessary for the immunological control of developing larvae. International Archives Allergy Applied Immunology 48, 211-219.

Love, R.J. and Ogilvie, B.M. (1977) Nippostrongylus brasiliensis and Trichinella spiralis: Localization of Lymphoblasts in the small intestine of Parasitized Rats. Experimental Parasitology 41, 124-132.

Love, R.J., Ogilvie, B.M. and McLaren, D.J. (1976) The immune mechanism which expels the intestinal stage of Trichinella spiralis from rats. Immunology 30, 7-15.

MacDonald, T.T., Murray, M. and Ferguson, A. (1980) Nippostrongylus brasiliensis: Mast Cell Kinetics at Small Intestinal Sites in Infected Rats. Experimental Parasitology 49, 9-14.

Manson-Smith, D.F., Bruce, R.G. and Parrott, D.M.V. (1979a) Villous Atrophy and expulsion of intestinal Trichinella spiralis are mediated by T cells. Cellular Immunology 47, 285-292.

Manson-Smith, D.F., Bruce, R.G., Rose, M.L. and Parrott, D.M.V. (1979b) Migration of lymphoblasts to the small intestine III Strain differences and relationship to distribution and duration of Trichinella spiralis infection. Clinical Experimental Immunology 38, 475-482.

Meerovitch, E. and Ackerman, S.J. (1974) Trypanosomiasis in rats with Trichinosis. Transactions Royal Society Tropical Medicine Hygiene 68, 417.

Miller, H.R.P. (1980) Expulsion of Nippostrongylus brasiliensis from rats protected with serum. I The efficacy of sera from singly and multiply infected donors related to time of administration and volume of serum injected. Immunology 40, 325-334.

Miller, H.R.P. and Nawa, Y. (1979) Nippostrongylus brasiliensis: Intestinal Goblet-cell response in adoptively immunized rats. Experimental Parasitology 47, 81-90.

Miller, T.A. (1965) Persistence of immunity following double vaccination of pups with X-irradiated Ancylostoma caninum larvae. Journal of Parasitology 51, 705-711.

Miller, T.A. (1967) General principles involved in the use of irradiated larval vaccines. Reprint from Radiosterilization of Medical Products 219-230 IAEA Vienna.

Miller, T.A. (1971) Vaccination against Canine Hookworm Diseases. Advances In Parasitology 9, 153-183.

Miller, T.A. (1978) Industrial Development and Field Use of the Canine Hookworm Vaccine. Advances in Parasitology 16, 333-342.

Mitchell, G.F. (1979) Review: Effector cells, molecules and mechanisms in host-protective immunity to parasites. Immunology 38, 209-223.

Molinari, J.A., Cypess, R.H. and Ebersole, J.L. (1974) Effect of Trichinella spiralis infection on the cell mediated immune response to BCG. International Archives Allergy Applied Immunology 47, 483-487.

Molinari, J.A., Ebersole, J.L. and Cypess, R.H. (1978) Specific antibody levels in the serum of Heligmosoroides polygyrus-infected mice. Journal of Parasitology 64, 233-238.

Moore, A.R. and Hall, J.G. (1973) Non-specific entry of thoracic duct immunoblasts into intradermal foci of antigens. Cellular Immunology 8, 112-119.

Moqbel, R. (1977) Studies on the Host-Parasite Relationship of Strongyloides ratti in Rats. PhD. Thesis. University of London.

Moqbel, R. (1980) Histopathological changes following primary, secondary and repeated infections of rats with S. ratti, with special reference to tissue eosinophils. Parasite Immunology 2, 11-27.

Moqbel, R. and Wakelin, D. (1979) Trichinella spiralis and Strongyloides ratti: Immune interaction in Adult Rats. Experimental Parasitology 47, 65-72.

Moqbel, R. and Wakelin, D. (1981) Immunity to Strongyloides ratti in rats. I Adoptive transfer with mesenteric lymph node cells. Parasite Immunology (in press).

Movsesijan, M. (1973) Immunisation in Dictyocaulus filaria infection; in, "Helminth Diseases of Cattle, Sheep and Horses in Europe" ed. Urquhart, G.M. and Armour, J., p. 74-78. R. MacLehose and Co. Ltd. The University Press, Glasgow.

Mulligan, W., Gordon, H.McL., Stewart, G.D.F. and Wagland, B.M. (1961) The use of irradiated larvae as immunizing agents in Haemonchus contortus and Trichostrongylus colubriformis infections of sheep. Australian Journal of Agricultural Research 12, 1175-1187.

Nash, D.R. and Heremans, J.F. (1969) A Quantitative Antibody-Binding Method for the determination of specific Antibody within different Immunoglobulin classes. Application to four Ig Classes in the mouse. Immunology 17, 685-694.

Nawa, Y. and Miller, H.R.P. (1978) Protection against Nippostrongylus brasiliensis by adoptive immunization with immune thoracic duct lymphocytes. Cellular Immunology 37, 51-60.

Nawa, Y. and Miller, H.R.P. (1979) Adoptive transfer of the intestinal mast cell response in rats infected with Nippostrongylus brasiliensis. Cellular Immunology 42, 225-239.

Nawa, Y., Parish, C.R. and Miller, H.R.P. (1978) The protective capacities of fractionated Immune thoracic duct lymphocytes against Nippostrongylus brasiliensis. Cellular Immunology 37, 41-50.

Nelson, G.S. (1966) Discussion, In: Webbe and Jordan: Recent advance in Knowledge of Schistosomiasis in East Africa. Transactions Royal Society Tropical Medicine and Hygiene 60, 279-312.

Normann, S.J. and Schardt, M. (1978) A Macrophage inflammation test using subcutaneous nitrocellulose filters. Journal Reticuloendothelial Society 23, 153-160.

Ogbourne, C.P. and Duncan, J.L. (1977) Strongylus vulgaris in the horse: its biology and veterinary importance. Miscellaneous Publication No. 4, 31-33. Commonwealth Agricultural Bureaux.

Ogilvie, B.M., Askenase, P.W. and Rose, M.E. (1980) Basophils and eosinophils in three strains of rats and in athymic (nude) rats following infection with the nematodes Nippostrongylus brasiliensis or Trichinella spiralis. Immunology 39, 385-389.

Ogilvie, B.M. and Jones, V.E. (1968) Passive protection with cells or antiserum against Nippostrongylus brasiliensis in the rat. Parasitology 58, 939-949.

Ogilvie, B.M. and Jones, V.E. (1971a) Immunity in the parasitic relationship between helminths and hosts. Progress in Allergy 17, 93-144.

Ogilvie, B.M. and Jones, V.E. (1971b) Nippostrongylus brasiliensis: A review of immunity and the Host-Parasite relationship in the rat. Experimental Parasitology 27, 138-177.

Ogilvie, B.M. and Love, R.J. (1974) Co-operation between antibodies and cells in immunity to a nematode parasite. Transplantation Reviews 19, 147-168.

LOVE, R.J. and OGILVIE, B.M. (1974) The immune deficiency responsible for the persistence of infection in neonatal rats infected with Nippostrongylus brasiliensis. Parasitology 69, XXI.

Ogilvie, B.M., Love, R.J., Jarra, W. and Brown, K.N. (1977) Nippostrongylus brasiliensis infection in rats. The cellular requirement for worm expulsion. Immunology 32, 521-528.

Othuman, P., Denham, D.A., McGreevy, P.B., Nelson, G.S. and Rogers, R. (1979) Successful vaccination of cats against Brugia pahangi with larvae attenuated by irradiation with 10 k.rad cobalt 60. Parasite Immunology 1, 209-216.

Panter, H.C. (1969a) Host-parasite relationships of Nematospiroides dubius in the mouse. Journal of Parasitology 55, 33-37.

Panter, H.C. (1969b) The mechanism of immunity of mice to Nematospiroides dubius. Journal of Parasitology 55, 38-43.

Parrott, D.M.V. and Ferguson, A. (1974) Selective migration within the mouse small intestine. Immunology 26, 571-588.

Peacock, R. and Poynter, D. (1979) Field experience with a bovine lungworm vaccine. In Symposia British Society for Parasitology 18, 141-148.

Peters, W. (1978) The relevance of Parasitology to Human Welfare Today - Medical Aspects - Comments and Discussion II. Symposia British Society for Parasitology 16, 25-40.

Piller, N.B. (1978) A morphological assessment of the stimulatory effect of Coumarin on macrophages. British Journal Experimental Pathology 59, 93-96.

Portaro, J.K., Britton, S. and Ash, L.R. (1976) Brugia pahangi: depressed mitogen reactivity in filarial infections in the jird, Meriones unguiculatus. Experimental Parasitology 40, 438-446.

Pross, H.F. and Eidinger, D. (1974) Antigenic Competition: A review of nonspecific Antigen-induced suppression. Advances in Immunology 18, 133-168.

Prowse, S.J., Ey, P.L. and Jenkin, C.R. (1978a) Immunity to Nematospiroides dubius: Cell and immunoglobulin changes and the onset of immunity in mice. Australian Journal Experimental Biology and Medical Science 56, 237-246.

Prowse, S.J., Mitchell, G.F., Ey, P.L. and Jenkin, C.R. (1978b) Nematospiroides dubius: susceptibility to infection and the development of resistance in hypothymic (nude) Balb/c mice. Australian Journal Experimental Biology Medical Science 56, 561-570.

Prowse, S.J., Mitchell, G.F., Ey, P.L. and Jenkin, C.R. (1979) The development of resistance in different inbred strains of mice to infection with Nematospiroides dubius. Parasite Immunology 1, 277-288.

Rau, M.E. and Tanner, C.E. (1975) BCG suppresses growth and metastasis of hydatid infections. Nature 256, 318-319.

Richards, A.J., Bryant, C., Kelly, J.D., Windon, R.G. and Dineen, J.K. (1977) The metabolic lesion in Nippostrongylus brasiliensis induced by postaglandin E_1 in vitro. International Journal for Parasitology 7, 153-158.

Rickard, M.D. and Adolph, A.J. (1978) Vaccination of Calves against Taenia saginata infection using a Parasite-Free vaccine. Veterinary Parasitology 1, 389-392.

Rosales, C.L., Bennett, J.M. and Rutenburg, A.M. (1966) Histochemical Demonstration of Leucocyte Acid Phosphate in Health and in Disease. British Journal Haematology 12, 172-177.

Rose, M.E., Ogilvie, B.M., Hesketh, P. and Festing, M.F.W. (1979) Failure of nude (athymic) rats to become resistant to reinfection with the intestinal coccidian parasite Eimeria nieschulzi or the nematode Nippostrongylus brasiliensis. Parasite Immunology 1, 125-132.

Rose, M.L., Parrott, D.M.V. and Bruce, R.G. (1976a) Migration of lymphoblasts to the small intestine I. Effect of Trichinella spiralis infection on the migration of mesenteric lymphoblasts and mesenteric T lymphoblasts in syngeneic mice. Immunology 31, 723-730.

Rose, M.L., Parrott, D.M.V. and Bruce, R.G. (1976b) Migration of lymphoblasts to the small intestine II. Devergent migration of mesenteric and peripheral immunoblasts to sites of inflammation in the mouse. Cellular Immunology 27, 36-46.

Rothwell, T.L.W. (1978) Vaccination against the nematode Trichostrongylus colubriformis. III. Some observations on factors influencing immunity to infection in vaccinated guinea-pigs. International Journal for Parasitology 8, 33-37.

Rothwell, T.L.W., Dineen, J.K. and Love, R.J. (1971) The role of pharmacologically-active amines in resistance to Trichostrongylus colubriformis in the Guinea-pig. Immunology 21, 925-938.

Rothwell, T.L.W. and Huxtable, C.R. (1976) Basophil leucocytes in cutaneous hypersensitivity reactions in nematode-infected Guinea-pigs. Australian Journal Experimental Biology Medical Science 54, 329-335.

Rubin, R., Leuker, D.C. and Andersen, S. (1968) Protection of Mice against Nematospiroides dubius by subcutaneously administered larval vaccines. Journal of Parasitology 54, 1237-1238.

Rubin, R., Leuker, D.C., Flom, J.C. and Andersen, S. (1971) Immunity against Nematospiroides dubius in CFW Swiss Webster Mice protected by subcutaneous larval vaccination. Journal of Parasitology 57, 815-817.

Ruitenberg, E.J., Elgersma, A., Kruizinga, W. and Leenstra, F. (1977) Trichinella spiralis infection in congenitally athymic (nude) mice. Parasitological, serological and haematological studies with observations on intestinal pathology. Immunology 33, 581-587.

Sarles, M.P. and Taliaferro, W.H. (1936) The local points of Defense and the passive transfer of acquired immunity to Nippostrongylus muris in rats. Journal Infectious Diseases 59, 207-220.

Schlossman, S.F., Levin, H.A., Rocklin, R.E. and David, J.R. (1971) The compartmentalization of antigen-reactive lymphocytes in desensitized Guinea-pigs. Journal Experimental Medicine 134, 741-750.

Selby, G.R. and Wakelin, D. (1973) Transfer of immunity against Trichuris muris in the mouse by serum and cells. International Journal for Parasitology 3, 717-722.

Semrad, J.E. (1937) Effects of Roentgen radiation on trichinosis in the albino rat. American Journal Roentgenology 38, 470-477.

Shimp, R.G., Crandall, R.B. and Crandall, C.A. (1975) Heligmosomoides polygyrus (= Nematospiroides dubius): suppression of antibody response to orally administered sheep erythrocytes in infected mice. Experimental Parasitology 38, 257-269.

Skamene, E., Chayasirisobhan, W. and Kongshavn, P.A.L. (1978) Increased phagocytic activity of splenectomized mice challenged with Listeria monocytogenes. Immunology 34, 901-908.

Skrjabin, K.I., Shikhobalova, N.P. and Schulz, R.S. (1954) [Principles of Nematodology, ed. by K.I. Skrjabin. Vol. IV. Dictyocaulidae, Heligmosomatidae and Ollulanidae of animals]. Moscow: Izd. Akad. Nauk SSSR, 323p. In Russian, quoted Forrester, (1971) Journal of Parasitology 57, 498-503.

Sokal, R.R. and Rohlf, (1969) In Biometry, p. 240. W.H. Freeman & Co., San Francisco.

Solomon, M.S. and Haley, A.J. (1966) Biology of the rat nematode Nippostrongylus brasiliensis (Travassos, 1914). V. Characteristics of N. brasiliensis after serial passage in the laboratory mouse. Journal of Parasitology 52, 237-241.

Spurlock, G.M. (1943) Observations on host-parasite relations between laboratory mice and Nematospiroides dubius, Baylis. Journal of Parasitology 29, 303-311.

Stoner, R.D. and Hale, W.M. (1952) Effect of cobalt 60 Gamma radiation on susceptibility and immunity to Trichinosis. Proceedings Society Experimental Biology Medicine 80, 510-512.

Stromberg, B.E. and Soulsby, E.J.L. (1977) Ascaris suum: immunization with soluble antigens in the guinea pig. International Journal for Parasitology 7, 287-291.

Taliaferro, W.H. and Sarles, M.P. (1939) The cellular reactions in the skin, lungs and intestine of normal and immune rats after infection with Nippostrongylus muris. Journal Infectious Diseases 64, 157-192.

Taylor, M.G., James, E.R., Bickle, Q.E., Doenhoff, M.J., Nelson, G.S., Hussein, M.F. and Bushara, H.O. (1977) Vaccination in Schistosomiasis in Immunity in parasitic diseases. I:N.S.E.R.M. 72, 291-306.

Taylor, M.G., James, E.R., Nelson, G.S., Bickle, Q.D., Dunne, D.W. and Webbe, G. (1976) Immunisation of sheep against Schistosoma mattheii using either irradiated cercariae or irradiated schistosomula. Journal of Helminthology 50, 1-9.

Terry, R.J. (1977) Immunodepression in parasite infections in Immunity in parasitic diseases. I:N.S.E.R.M. 72, 161-178.

The Eosinophil (1980) Symposium. Transactions Royal Society Tropical Medicine and Hygiene. (in press).

Thompson, R.C.A. (1976) Inhibitory effect of BCG on development of secondary hydatid cysts of Echinococcus granulosis. Veterinary Record 99, 273.

Tyzzer, E.E. and Honeij, J.A. (1916) The effects of radiation on the development of Trichinella spiralis with respect to its application to the treatment of other parasitic diseases. Journal of Parasitology 3, 43-56.

Urquhart, G.M. (1977) Irradiated vaccines in Immunity in Parasitic diseases. I.N.S.E.R.M. 72, 263-274.

Urquhart, G.M., Mulligan, W., Eadie, R.M. and Jennings, F.W. (1965) Immunological studies on Nippostrongylus brasiliensis in the Rat: The Role of Local Anaphylaxis. Experimental Parasitology 17, 210-217.

Van Zandt, P.D. (1962) Immunity relationships in white mice infected with Nematospiroides dubius Baylis, 1926 (Nematoda: Heligmosomidae). II. Artificial immunization with antigen prepared from larvae. Journal of Parasitology 48, 249-251.

Wakelin, D. (1975a) Immune expulsion of Trichuris muris from mice during a primary infection: analysis of the components involved. Parasitology 70, 397-405.

Wakelin, D. (1975b) Genetic control of immune responses to parasites: immunity to Trichuris muris in inbred and random-bred strains of mice. Parasitology 71, 51-60.

Wakelin, D. (1978) Immunity to intestinal parasites. Nature 273, 617-620.

Wakelin, D. (1980) Genetic control of immunity to parasites. Infection with Trichinella spiralis in inbred and congenic mice showing rapid and slow responses to infection. Parasite Immunology 2, 85-98.

Wakelin, D. and Donachie, M. (1980) Genetic control of immunity to parasites: adoptive transfer of immunity between inbred strains of mice characterized by rapid and slow immune expulsion of Trichinella spiralis. Parasite Immunology 2, (in press).

Wakelin, D. and Lloyd, M. (1976a) Immunity to primary and challenge infections of Trichinella spiralis in mice: a re-examination of conventional parameters. Parasitology 72, 173-182.

Wakelin, D. and Lloyd, M. (1976b) Accelerated expulsion of adult Trichinella spiralis in mice given lymphoid cells and serum from infected donors. Parasitology 72, 307-315.

Wakelin, D. and Selby, G.R. (1976) Immune expulsion of Trichuris muris from resistant mice: supporession by irradiation and restoration by transfer of lymphoid cells. Parasitology 72, 41-50.

Wakelin, D. and Wilson, M.M. (1979) T and B cells in the transfer of immunity against Trichinella spiralis in mice. Immunology 37, 103-109.

Wakelin, D. and Wilson, M.M. (1980) Immunity to Trichinella spiralis in irradiated mice. International Journal for Parasitology 10, 37-41.

Waksman, B.H. (1977) Tolerance, the thymus and suppressor T cells. Clinical Experimental Immunology 28, 363-374.

Weiss, N. (1978) Dipetalonema viteae: 'In vitro' blastogenesis of hamster spleen and lymph node cells to phytohaemagglutinin and filarial antigens. Experimental Parasitology 46, 283-299.

Wescott, R.B. and Todd, A.C. (1966) Adaptation of Nippostrongylus brasiliensis to the mouse. Journal of Parasitology 52, 233-236.

Wilson, R.J.M. and Bloch, K.J. (1968) Homocytotropic antibody response in the rat infected with the nematode Nippostrongylus brasiliensis II. Characteristics of the immune response. Journal of Immunology 100, 622-628.

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